Akademiya Nauk SSSR

LIFE PHENOMENA
A Historical Survey

NSLATED FROM RUSSIAN

Published for the National Aeronautics and Space Administration, U.S.A.
and the National Science Foundation, Washington, D.C.
by the Israel Program for Scientific Translations

AKADEMIYA NAUK SSSR. INSTITUT ISTORII ESTESTVOZNANIYA
I TEKHNIKI

Academy of Sciences of the USSR. Institute of the History
of Natural Science and Technology

LIFE PHENOMENA
A Historical Survey

(Fizicheskie i khimicheskie osnovy zhiznennykh yavlenii)
(Istoricheskie ocherki)

Izdatel'stvo Akademii Nauk SSSR
Moskva 1963

Translated from Russian

Israel Program for Scientific Translations
Jerusalem 1966

NASA TT F-381
TT 66-51018
Published Pursuant to an Agreement with
THE NATIONAL AERONAUTICS AND SPACE ADMINISTRATION, U.S.A.
and
THE NATIONAL SCIENCE FOUNDATION, WASHINGTON, D.C.

Copyright © 1966
Israel Program for Scientific Translations Ltd.
IPST Cat. No. 1660

Translated and Edited by IPST Staff

Printed in Jerusalem by S. Monson

Price: $ 5.00

Available from
U.S. DEPARTMENT OF COMMERCE
Clearinghouse for Federal Scientific and Technical Information
Springfield, Va. 22151

xi/13

TABLE OF CONTENTS

Introduction 1

Blyakher, L. Ya. The Development of Notions on the Material Basis of Living Structures, from Antiquity to the Beginning of the 20th Century 3

Belousov, L. V. Origin, Development and Prospects of the Theory of the Biological Field 45

Grigoryan, N. A. The Birth of Electrocardiography in Russia 92

Senchenkova, E. M. V. N. Lyubimenko's Studies of Chlorophyll and Their Modern Development 116

List of Abbreviations 171

INTRODUCTION

Modern biology has widely and effectively adapted physical and chemical methods for solving basic problems of form, structure, function, and development of plant and animal organisms.

The history of this application of physics and chemistry to biology is extremely instructive. It reveals the debt that biological science owes to the theoretical concepts and practical investigations of the past, as well as to modern technology, in its successful investigations of vital phenomena at the molecular and atomic levels. The present status and prospects of biology, can only be understood through this historical approach.

This collection is devoted to several problems in those fields of natural science which are rapidly developing along multi-disciplinary lines (physical chemical, and biological).

Several articles in the collection touch on questions of the material nature of vital phenomena which were posed in ancient times and have since been taken up at various stages of scientific development which depended upon the then current level of knowledge and technology. Other articles consider problems which arose in comparatively recent times, problems which could arise only at a definite stage of development of biological science. This study shows the development of new concepts from the scientific discoveries, technical inventions and especially the theoretical insights of the past.

L. Ya. Blyakher

THE DEVELOPMENT OF NOTIONS ON THE MATERIAL BASIS OF LIVING STRUCTURES, FROM ANTIQUITY TO THE BEGINNING OF THE 20th CENTURY

(Studies in the history of morphology. 5[*])

1. DOCTRINE ON THE DISCRETENESS OF PRIMAL MATTER IN ANTIQUITY

Early notions about the materiality of living organisms and their vital processes are lost in deep antiquity, merged with the materialist outlook of the ancients on nature as a whole.

The ancient cultures of Asia (India and China) and of Africa (Egypt) held the concept of a fundamental substance or substances as the basis of all natural bodies, animate and inanimate. Greek thinkers of the sixth to fifth century B.C. also believed in one or several primal substances, i.e., matter from which all bodies were derived.[1][**] Empedocles, like the ancient Indian materialists, considered fire, air, water, and earth to be the four primal elements.

The notion of the divisibility of matter into indivisible particles is associated with the name of a contemporary of Empedocles, Anaxagoras, who assumed the existence of infinitely small elements differing qualitatively and combining to provide the multiformity of natural bodies. Anaxagoras thought that these different elements or seeds (sperm), which were later to be called, probably by Aristotle, the uniform substances, enter into the composition of natural (including living) bodies, but in unequal quantities. We postulated that in flesh, for example there are more "flesh" particles than any other kind, while in bones, "bone" particles predominate. Much later, in the first century B.C., Lucretius, the Roman poet and materialist philosopher, in expounding Anaxagoras' theories on the uniform substances wrote:

> "... that bones are formed of minute miniature bones,
> flesh of minute miniature morsels of flesh,
> blood by the coalescence of many drops of blood."[2]

---

[*] Published under the following titles: Ot "pozvonochnoi teorii cherepa" k ucheniyu o metamerii golovy pozvonochnykh (From the "Vertebral Theory of the Skull" to the Teaching on Metamerism of the Head of Vertebrates). — Ann. biol. Mosk. Obshch. Ispyt. Prir., Vol. 1:155-264, 1959; Vozniknovenie kletok v ontogeneze (The Origin of cells in Ontogenesis). — Trudy Instituta istorii estestvoznaniya i tekhniki, Vol. 32: 3-57, 1960; Tak nazyvaemaya idealisticheskaya morfologiya i ee mesto v istorii morfologicheskikh nauk (The SO-called Idealist Morphology and Its Place in the History of Morphological Sciences). Loc. cit., Vol. 36:3-52, 1961; Sootnoshenie formy i funktsii (The Relationship of Form and Function). Loc. cit., Vol. 40: 118-156, 1962.

[**] [For this and other footnotes appearing in this paper, see "Notes" at the end of this paper.]

Anaxagoras did not believe that there existed any vacuum separating the uniform substances and he assumed the infinite divisibility of matter

> "At the same time he does not admit to any vacuum in things,
> Or to any limit to the splitting of matter. . ."[3]

Opposed to Anaxagoras' idea of the diverse infinitely divisible uniform substances was the doctrine of Leucippus and Democritus on the uniformity of particles of matter (atoms), divisible only up to a certain limit. According to Democritus, the multiformity of bodies depends not on the differences in kind, of component atoms, but on the quantitative differences in the shape of the atoms, on their configuration and combination and on the position of each individual atom. The movement of particles, according to Democritus, is causally determined, although causality or necessity had for him an abstract character of strict determinism. Democritus thought that the matter of different organs possesses specific characteristics and that this matter enters into the seed from the organs and thus assures the repetition of parental characteristics in offspring. Hippocrates developed similar notions. Epicurus characterized weight as another difference among the indivisible particles of matter. In Engels' opinion this signified a new stage of ancient materialism.[4] Unlike Democritus, who assumed that atoms can have an angular form with sharp and curved projections, Epicurus thought that atoms are characterized by a round form and smooth surface.

The notion was thus gradually worked out that the qualitative peculiarities of natural bodies, in particular of organized bodies, depend upon the characteristics of the matter composing them: specifically, on the mixture in fixed proportions of the different particles, or on the dissimilar spatial arrangements, or on the relationships of the homogeneous units or atoms to each other.

Ancient materialism at the end of the third to the beginning of the second century B.C. was fiercely opposed by idealism, which was most persistently and stubbornly championed by Plato. According to Diogenes Laertius, "Plato wanted to burn all the works of Democritus."[5] Following Socrates' view that only esthetic [sic] problems and not the study of tangible things are deserving of the philosopher's attention, Plato set up what he regarded as being the world of abstract ideas — against nonexistence, i.e., the material world. The real world, i.e., the world of ideas, is inaccessible to the senses, according to Plato, who said "The eternal, colorless and formless essence is observable only by the reason."[6]

Aristotle retained in his world view the central concept of his teacher, Plato,— the concept of essence, of being, of individual existence as such. In Aristotle's opinion this concept becomes clearer as one studies organized bodies and their component parts. "Therefore we use the word essence, for animals, plants and their parts and also for natural bodies — fire, water and soil and for all their varieties and for all their components."[7] In polemics with the materialist philosophers Aristotle refused to consider matter the essence of natural bodies. "Now the substratum is that of which everything else is predicated, while it is itself not predicated of anything else."[8] ... Now those who were the first to study Nature in the early days spent their time in trying to discover what the material principle of the material cause was and what it was like; ... assuming throughout that the underlying material had, by necessity, some definite nature: e.g., that the nature of Fire was hot and light; of Earth, cold and heavy. At any rate, that is how they actually explain the formation of the world order. In a like manner they describe the formation of animals and plants, saying that the stomach and every kind of receptacle for food and for residue is formed by the water flowing in the body and the nostril openings are forcibly made by the passage of the breath. Air and water, of course, according to them are the material of which the body is made: they all say that Nature is composed of substances of this sort."[9]

Aristotle thought otherwise: "It is not enough to state simply the substances out of which they are made, as 'out of fire,' or 'out of earth. If we were describing a bed or any other like article, we should endeavor to describe the form of it rather than the matter (bronze or wood) — or, at any rate, the matter, if described, would be described as belonging to the concrete whole. For example, a 'bed' is a certain form in certain matter, or, alternatively, certain matter that has a certain form; so we should have to include its shape and the manner of its form in our description of it. And in fact the 'formal' nature is of more fundamental importance than the 'material' nature."[10] Only matter combined with form can be recognized as the essence of natural and artificial bodies.[11]

In clarifying these notions on the organization of living beings, Aristotle referred to the "threefold composition of animals." First of all, taking the four elements of Empedocles (earth, air, water and fire), Aristotle attributed sense qualities to them: warmth, cold, dryness and wetness. Earth, in his opinion, is cold and dry, water — cold and wet, air — warm and wet, fire — warm and dry. "It is just these four, the fluid substance, the solid, the hot and the cold, which are the matter of composite bodies; and the other differences and qualities — such as heaviness lightness, firmness loosness, roughness smoothness."[12] As the next step in his structure of animals, Aristotle visualized homogeneous parts of the body (flesh, bones, etc.) each part being built only from the qualitatively different elements just enumerated. Finally, the homogeneous parts (later to be called Tissues) combine into heterogeneous groups, forming the anatomical parts of the body: face, hands, etc.

As a result of their diversity of structure and form, the heterogeneous parts (organs or parts of the body) consist of substances possessing differing physical characteristics. "One of the parts, for instance, will be soft, another hard, while one is fluid, another solid; one viscous, another brittle. In the nonuniform parts, on the other hand, these dynamics are found in combination, not singly."[13] Thus, Aristotle accepted a hierarchy of matter and of forms of a living body: elements form the matter of homogeneous parts, whose characteristics appear in a form related to these elements; in turn, the homogeneous parts form the matter of heterogeneous parts, whose characteristics then take the form of the heterogeneous parts.

Empedocles did not reduce the characteristics of animal parts to the characteristics of their composite elements. "Empedocles... is forced to assert that it is the logos which is a thing's essence or nature. For instance, when he is explaining what Bone is, he says not that it is any one of the elements, or any two, or three, or even all of them, but that it is 'the logos of the mixture' of the elements."[14] Aristotle acknowledged that "primal matter" is something unspecific, without any form; therefore, it is not perceived by the senses and, in the final account, is incognizable.[15] From Aristotle's viewpoint, the origin of the existence of each body possessing individual characteristics lies in its essence and does not depend upon the peculiarities of its composite matter. "The process is for the sake of the actual thing, the thing is not the for sake of the process."[16] And further, "Because man is such and such, therefore the process of his formation must be such and such and must take place in such a manner... And similarly with all the things that are constructed by Nature."[17] Thus, according to Aristotle, it is not the characteristics of the elements which determine the characteristics of the whole formed by them in combination; rather, it is the whole originating in the elements which determines their realization.

Since Aristotle was sure that the whole cannot be completely reduced to its parts, it follows that the assumed characteristics of specific material elements were irrelevant to his theory. "Since that which is compounded out of something so that the whole is one, not like a heap but like a syllable — now the syllable is not its elements, ba is not the same as b and a, nor is flesh, fire and earth... not only its elements... but also something else... and that it is the cause which makes this thing flesh and that a syllable... And similarly in all other cases. And, this is the substance of each thing (for this is the primary cause of its being)..."[18], e.g., a and b are the element of the syllable.

Titus Lucretius Carus revived the idea of atomism, developed by Democritus and Epicurus, in the poem "On the Nature of Things." He denied Democritus' ideas on the inevitable necessity of all natural phenomena and recognized spontaneous deviations in the movement of atoms analogous to man's relatively free will. Taking up Democritus' ideas on the form of atoms, Lucretius conjectured that atoms can have sharp, hooked extensions by which they are coupled to each other. The atoms which thus form organized bodies are, in Lucretius' opinion, indestructible and multiform. Animate bodies consist of the same elementary particles that are present in inanimate bodies, but combined in different ways and quantities.

> 'Obviously, it is because there are in things many elements
> common to many things commingled in many ways...
> It often makes a big difference in what combinations and
> positions the selfsame elements occur, and what motions
> they mutually pass on or take over."[19]

The number of primal substances, however, and the number of possible combinations of different atoms are limited.

> "Within the limits of any single particle,
> there can only be a limited range of forms."[20]
> "... So in other things, although many atoms are
> common to many substances, yet these substances
> may still differ in their composition."[21]
> "It must not be assumed that atoms of every sort
> can be linked in every variety of combination."[22]

2. NOTIONS ON THE DISCRETENESS OF MATTER IN ANIMATE BODIES DURING THE 17th AND 18th CENTURIES

After the centuries-old supremacy of medieval scholasticism, the revival of experimental natural science brought about deterministic and mechanistic views of nature. F. Bacon was the herald of these views in the 17th century. R. Descartes advanced the theory of the material nature of the world with respect to all natural phenomena except the soul of man, and Hobbes and Gassendi tried to explain the human psyche in mechanistic terms. Hobbes and Descartes (particularly the latter) attempted an explanation of natural phenomena by applying mathematical (geometrical) and physical (mechanical) principles. Matter in an infinite universe, in Descartes' philosophy, was originally homogeneous and only divided through a process of vertical movement into three elements: earth, air and fire. These elements differed in the diminishing sizes of particles or corpuscles which, in principle, were divisible to infinity. Descartes tried to explain physiological phenomena by the movement of the corpuscles, movement as constant and indestructible as the corpuscles themselves. Gassendi returned to the notions of Democritus and Epicurus about invisible particles of matter — atoms, differing in form, magnitude and weight.

Jean Meslier's famous "Testament" became popular in the 1830's. He thought that only matter exists, containing within itself the source of movement. "The combination and separation of matter," wrote Meslier, "is only the natural result of its movement and the regular or irregular movement of its parts.[23] The French materialists of the 18th century, particularly Lamettrie and Diderot, followed in Meslier's footsteps. Like Meslier, Lamettrie thought that "matter contains in itself the moving force animating it, which is at the bottom of all laws of movement."[24] In Diderot's opinion, "a molecule possesses the characteristic of being, itself an active force. It acts on another molecule, which in turn acts on the first."[25] "I conclude that matter is heterogeneous, that an infinite quantity of diverse elements exist in nature."[26]

In the middle of the 18th century, M. V. Lomonosov developed a progressive notion of the unity of matter and movement. He presented an atomic-molecular hypothesis of the structure of organic and inorganic matter based on empirical data. Both types of matter, in his opinion, consist of similar (inorganic) substances, but animate beings differ from inanimate bodies in the combination of their chemical parts. "In organic (bodies), "wrote Lomonosov, "the bodily parts are constructed and connected with each other in such a way that the cause of one part is included in the other connected with it... Although the organs of animals and plants are very fine, they consist of even finer particles, specifically of inorganic, i.e., compound bodies, because, as a result of chemical operations, their organized structure is destroyed and compound bodies are formed from them. Thus, all compound bodies which are derived from animal or plant bodies by natural or artificial means also consist of chemical matter."[27]

In the 18th century Buffon and Maupertuis revived speculative notions similar to those of Lucretius and other ancients about the particles from which living organisms are constructed. However, while Lucretius saw no difference between the particles forming inanimate and animate bodies, Buffon supposed that animate bodies consist of "living molecules" as distinct by nature from molecules of inanimate bodies.[28] These "living molecules" form the physical basis of life and are indestructible even after the death of the organism. (Lomonosov's view was more progressive since it did not lead to an idealistic conflict between opposing notions of the nature of animate versus inanimate matter).

P. Maupertuis also posited the existence of particles specific to life to explain the similarity of offspring to parents. According to Maupertuis, seed fluids of the father and mother consist of a multitude of particles ("embryos") formed in every organ and have the same nature as the organ from which they originate. The paternal and maternal "embryos" combine to produce the offspring, which reproduces the characteristics of both parents. He foresaw a possibility of verifying experimentally the inheritance of mechanical injuries. "If over a long period of time," he wrote, "each generation of certain animals is maimed, it may be discovered that the excised parts will diminish little by little and perhaps will finally disappear."[29]

Erasmus Darwin acknowledged the contribution of Buffon's theories to his formulation of the doctrine on the structure of living bodies. Developing an idea which Hippocrates had expressed and which Aristotle in turn had criticized, Darwin proposed that the male contains in his sexual organs "... 'fibers,' and the female, 'molecules,' the primordia being transferred from all the somatic organs into the sex 'glands'."[30]

3. THE DISCOVERY OF CELLS. THE FIRST ATTEMPTS TO EXPLAIN THE STRUCTURE OF CELLS AND THEIR CHEMICAL COMPOSITION

It was inevitable that a priori notions about particles ("living molecules," "embryos" or "fibers") would sooner or later clash with results of empirical research. In the 17th and 18th centuries the structure of living beings was systematically studied under the microscope. The weak optical systems in the microscopes of that time did not permit reliable observation of cell, tissue or organ structure. R. Hook described plant cells as empty or porous, and, for a long time afterward, the cell content was at best thought of as a homogeneous liquid. Contemporaneously with Hook, Malpighi and Grew observed formations similar to "cells" in microscopic plant sections and called them "saccules" or "vesicles." Somewhat later Leuwenhoek described red corpuscles and spermatozoa of animals. In the middle of the 18th century K. F. Wolff wrote that plant buds and chicken embryos consist of "granules" or "vesicles." Plant and animal growth and development occurs according to Wolff, because a current of nutritive liquids produces an increase in the size of these bubbles. The bubbles then take on round or polygonal shapes or are stretched into tubular or vessel-like forms.

The majority of researchers in the 18th century were limited, according to Wolff, by the then-current notions of cells, as cavities or structureless grains or bubbles. And yet, it was in the second half of the 18th century that the first notions on the internal structure of cells were formulated by the physiologist Fontana. His observations were far in advance of his time and anticipated the microscopists of the future by whom they were to be confirmed many years later. His paper on animal and vegetal poisons included anatomical descriptions and theories of the structure of animal cells. In a paragraph devoted to microscopic observations of the cutaneous mucus of the eel, Fontana recorded seeing nuclei and "even nucleoli" in cells ("bubbles"). It is obvious that he did not understand the full meaning and significance of his observations: "I allowed them (the bubbles) to dry on the glass, after which they appeared more irregular than before, with broad, transparent margins on all sides, and in each, an irregular body was disposed in a different part of the bubble. I crushed two or three such bubbles, and a great number of very small bodies came out from them."[31] Fontana also noted the granular content of fat cells, and specific features of the inner structure of nerve cells. His descriptions of cell structure were neither understood nor appreciated in his time. As late as the beginning of the 19th century, totally abstract representations of the microscopic structure of organized bodies were still widely accepted. For example, in the "Lehrbuch der Naturphilosophie" of L. Oken, organisms were described as an accumulation of particles which the author called "organic crystals," "slimy vesicles," "organic dots," "galvanic vesicles," and even "infusoria."

The development of the achromatic microscope and the gradual improvement of its optical possibilities permitted more accurate study of cell structure, especially in plants, and the first observation of the cell's most noticeable structural feature — the cell membrane — followed. The cells nucleus, which Fontana had been the first to observe, was rediscovered in the first half of the 19th century in an unhatched chicken egg (J. E. Purkyně)[32] and in plant cells (Mirbel).[33] R. Brown[34] demonstrated that the nucleus is an obligatory structural part of any cell. Purkyně was the first to work with animal tissues that had been condensed, dyed and clarified with reagents. He and his students described nuclei with nucleoli in nerve cells ("ganglionic kernels"), in glandular stomach cells, in the epidermis and in internal organs (spleen and thyroid glands).[35] The terms, "nucleus" and "nucleolus," were introduced by a student of Purkyně's, G. Valentin.

The works of F. Meyen[36], Th. Schwann[37] and, particularly, M. Schleiden[38] are largely concerned with the cell nucleus, which Schleiden called "cytoblast" (cell former). He proposed a theory of cell regeneration by nuclei which is in error.

F. Dujardin was one of the first to devote attention to the distinctive substance forming the cell content. In dealing with the structure of protozoa, Dujardin noted that he failed to discover in them organs similar to those of higher animals. He particularly noted the absence of the numeous stomachs which C. Ehrenberg attributed to protozoa. Dujardin summed up his observations as follows: "I might have lost my nerve and stopped these investigations which did not correspond to the evidence of my own eyes, had I not fortunately solved the problem by discovering the characteristics of "sarcode."

"This is the name I propose for the sticky, transparent substance, called by other researchers 'living jelly,' which is found in all lower animals, connecting other structural elements, and characterized by its insolubility in water, the spherical masses into which it contracts and which adhere to dissecting neddles, and viscouslike mucus. Water gradually reduces the volume of sarcode, destroying its structure and finally leaving only formless traces of grainy substance. It can be distinguished from mucus, gelatine, etc. by its insolubility in potash and by its coagulability in nitric acid. In this latter respect and in its insolubility in water, it is similar to albumen, but differs from it, in that albumen is dissolved in potash. But the most amazing characteristic of sarcode is the spontaneous formation of vacuoli in its mass... filled with liquid."[39]

Dujardin saw and described sarcode in tissues of liver fluke in tapeworms, annelida and larvae, as well as in protozoa.

J. Purkyne termed the basic substance of the cell "protoplasm." He wrote: "In plant cells liquid and solid are spatially separate: the first is the inner content and the latter is the enveloping substance. In the animal formation nucleus however, the two substances remain mixed together. In plants, the clearest example of the very first formative stage is found in the cambium and in animals, it can be found in the protoplasm of the embryo. The jelly-like balls or kernels appear as elementary particles in an intermediate condition between liquid and solid."[40] The above quotation shows that Purkyně did not yet have a clear notion of the similarity of plant and animal cell content; he even used the concept of cell in different senses for the designation of structural units of plant and animal organisms. Only after the work of H. Mohl[41] did the term "protoplasm" become generally accepted.

The first data on the chemical nature of substances in organic structures is apparently associated with the name of F. Raspail[42] who is considered by modern histochemists, such as Pierce, to be the pioneer of histochemical research. In Pierce's words,[43] Raspail was the first to properly evaluate microscopic chemistry of tissues as a science. Raspail was successful in identifying separate substances in tissues under the microscope using iodine as a test for starch, hydrochloric acid, for carbohydrates, xanthroprotein and sulfuric acid for proteins, and litmus for acidity.

At about the same time Raspail's compatriot, the chemist, M. Chevreul, postulated an opposing concept of biological phenomena. Chevreul considered it " "premature and irrelevant from the point of view of physicochemical science. . . to assert that the causes of animate and inanimate phenomena are identical."[44] However, he thought it just as "irrelevant from the philosophical point of view to explain all phenomena of life by one or many vital forces which differ absolutely from those in inanimate nature."[45]

Chevreul stopped at a question unsolved in his time — how living beings reproduce remarkably constant structural and functional traits of the species. In essence, he raised an important problem, that of the self-reproduction of chemical structural parts of the organism specific for the species, Haeckel and Erlsberg made purely speculative attempts to solve this problem (see pp. 18-19). According to their hypotheses, complex organic molecules are formed alongside old molecules of the same substance and under the latter's influence. N. K. Kol'tsov later developed a similar notion but one based on new biochemical facts. His basic idea recently received serious theoretical backing and experimental corrobation (see p. 32). Chevreul and Raspail tried to translate theories of organic structures into chemical language. The physical, or more precisely the physicochemical aspect of vital phenomena remained outside their field of vision. It was not until the middle of the last century that A. Kölliker in noting the selective character of cell permeability, touched on one of the basic problems of cell morphophysiology.[46]

Early speculations on the chemical and physicochemical nature of organic structures arose in the period when the microscopic organization of the cell was still practically unknown, and they waited upon the development of a century in cell chemistry and physical chemistry for evaluation.

In the middle of the 19th century, only the most general views were expressed on the nature of cells as elementary structures of a complex organism. Among these R. Virchow's general conclusions, which formed the basis of his book, "Die Cellularpathologie," were undoubtedly significant. Although he exaggerated the independence of individual cells, Virchow stated an important idea when he wrote: "It is hardly possible to be more inclined to mechanical theories than I am in matters of details and in explanations of processes under investigation; but in my opinion one must not forget that, to whatever significant measure the complex processes occurring within the cell are dependent upon conditions not connected with its whole, the true activity, nevertheless, originates from the cell itself which is active only as long as it appears to us an independent and whole element."[47]

The concept of protoplasm as the bearer of vital properties is associated with the names of M. Schultze and E. Brücke. Schultze described the relationship between protoplasm, the nucleus and the cell membrane (whose presence was earlier considered a basic characteristic of the cell) in the following way: "The nucleus is surrounded by the cell substance — a viscous, semi-liquid protoplasm which is opaque, as if filled with protein and fat grains. Protoplasm thus consists of a hyaloid basic mass with the viscosity and granularity peculiar to all protoplasm. The outermost layer of the aggregate (protoplasm) most often consists only of a homogeneous, hyaloid basic mass... The cell membrane, however, is like the cell protoplasm in chemical composition."[48] Schultze defined the cell thus: "The cell is an aggregate of protoplasm inside of which lies the nucleus... The cell leads, so to speak, a life complete in itself, of which the protoplasm appears to be the primary bearer; the nucleus, however, also plays a significant role which is still practically unknown."[49]

Soon after Virchow, E. Brücke suggested the idea of the cell as an autonomous unit in an article aptly titled "Die Elementaorganismen." Brücke argued in the following way: "I call cells elementary organisms just as we now call elements substances which at this stage seem chemically irresolvable; and yet, the irresolvability of these substances is not yet adequately proven. We have even less right to assert that cells in their turn, do not consist of other, still finer organisms, although at this time there is no proof of this."[50]

Brücke spoke still more cautiously about the supposed inner cell structures seen under the microscope: "When they say 'the cell membrane is structureless, protoplasm is a homogeneous mass, etc.,' this ought to mean only that the cell membrane appears unstructured to us, protoplasm seems to us to be a homogeneous mass."[51]

With respect to the chemical substances which compose cells, Brücke wrote: "... the structure of molecules (of these substances) is very complex; their high atomic weight shows that they consist of numerous elements. We cannot, however, be satisfied simply with this concept of molecular cell structure. We cannot imagine that a living, growing cell has a homogeneous nucleus, homogeneous membrane, and contains a simple solution of protein, since we shall never find in this simple protein those phenomena which we call the life process. We are forced, therefore, to recognize that in the cell, above the molecular structure of organic combinations, there exists another more complex structure of another order. We call this organization."[52]

The popular article of Th. Huxley, "The Physical Basis of Life," played an important part in the widespread notions of the 1860's on the material basis of vital phenomena, although Huxley himself, possibly to mask his true views, stated that he intended "a philosophical protest against what is generally called materialism"[53]. Huxley's article begins, by the way, with a denial of the widely held conviction that life, although existing by means of matter, does not depend upon matter. Huxley affirmed the universal material basis of vital phenomena. Despite the diversity of living beings in function, form and component substances, all organisms have a particular unity, since protoplasm is the basis of life for all. The differences among dissimilar organisms depend, in Huxley's opinion, upon their varying functional capabilities. "In the lowest organism all parts are competent to perform all functions, and one and the same portion of protoplasm may successively take on the function of feeding, moving or reproducing apparatus."[54] In higher organisms, however, each part, in performing its one designated function, loses the ability for others. Referring to the investigations of chemists, Huxley noted the "striking uniformity of material composition in living matter"[55] and objected to the claims of skeptics that the composition of living matter cannot be determined by chemical investigation because the living matter inevitably perishes in the process of analysis. The similar characteristics of proteins taken from different organisms proves, in Huxley's opinion "the existence of a general uniformity in the character of protoplasm, or physical basis of life, in whatever group of living beings it is studied."[56]

In discussing whether living matter exists everywhere as indestructible and unchangeable molecules which, in various combinations, form various organisms, or whether living matter is formed from inorganic matter from which it differs only in the arrangement of atoms, Huxley unhesitatingly held the latter opinion. Protoplasm is the product of a synthesis of inorganic substances and, upon dying, it again breaks down into inorganic substances. More than that, "living protoplasm not only ultimately dies... but it is always dying, and, strange as the paradox may sound, could not live unless it died." "Living matter," continued Huxley, "is a veritable 'Peau de Chagrin,' and is somewhat the smaller for every vital act... But happily, the protoplasmic peau de chagrin differs from Balzac's in that it can be repaired and brought back to its full size, after every exertion."[57]

Claude Bernard developed a similar point of view in the 1870's. He wrote: "Vital properties are in reality nothing more than physicochemical properties of organized matter."[58] Two kinds of phenomena are necessary for life: vital creation, or organic synthesis, and death, or organic destruction. Claude Bernard expressed his conclusion about the correlation and interdependence of the processes of creation and destruction thus: "... every manifestation (constructive—L.B.) of processes in a living being is inevitably connected with organic destruction; this is exactly what I meant when I said elsewhere ("La Revue des Deux Mondes." Vol. 9. 1875): "Life is death."[59]

Leading naturalists and thinkers of the 1860's and 1870's, who approached organic phenomena dialectically (whether consciously or not) shared this same concept of vital phenomena which led to a most general definition of life. Engels' views were almost textually similar. He wrote, "Life is the capability of existence of protein bodies, and this capability of existence consists essentially of a continual autoregeneration of the composite chemical parts of these bodies."[60] In another place, referring to Hegel's opinion that "life as such carries within it the seed of death"[61] Engels wrote: "Already no physiology is considered scientific if it does not consider death as an essential element of life, the negation of life as being essentially contained in life itself, so that life is always thought of in relation to its necessary result, death, which is always contained in it in germ."[62]

The difference between Engels' views and those of C. Bernard and Huxley come to the following: having rejected mechanical, metaphysical, i.e., inconsistent materialism, Engels remained a materialist and uncompromisingly applied the principles of dialectical materialism, while Bernard, approaching essentially the same conception of vital phenomena, called his viewpoint physical vitalism.[63] Huxley began and ended the above cited article with the declaration that he was not a materialist, evidently not wanting to enter into direct conflict with the ideology generally accepted by English bourgeois society. This compromising manner of expression did not jibe, however, with his optimistic prognoses on the future progress of science. With unconcealed irony, Huxley spoke about those who feared the successes of materialistic natural science. In the conclusion of his article he wrote: "... the physiology of the future [will] gradually extend the realm of matter and law until it is coextensive with knowledge, with feeling and with action. The consciousness of this great truth weighs like a nightmare, I believe, upon many of the best minds of these days. They watch what they conceive to be the progress of materialism, in such fear and powerless anger as a savage feels, when, during an eclipse, the great shadow creeps over the face of the sun."[64]

4. HYPOTHESES ON THE NATURE OF PROTOPLASM AND OF BEARERS OF VITAL CHARACTERISTICS WITHIN THE CELL

In the second half of the 19th century, several attempts were made to explain or to picture, although hypothetically, the nature of the material substrata of vital phenomena. Hypotheses on the structure of protoplasm and on the life-bearing properties of its structual formations resulted naturally from attempts to establish a material basis for cell structure and activity and to understand how parental and ancestral traits are inherited through the ovum and especially through the immeasurably smaller spermatozoon. Another source of hypotheses was the difficulties encountered in the empirical study of inner structure of cells of the body and of plant and animal sexual elements. Prenatal observations did not reveal in cells, ova or spermatozoa that complex structure which would explain their complex activities. The methods of fixation and tinting were of little help in this respect, especially as well-founded doubts arose rather early about the relationship of structures seen in fixed and tinted preparations to formations actually existing in the live cell.

Biologists used various approaches in hypothesizing the nature of material bearers of life. Morphologists and physiologists often tried merely to connect manifestations of vital cell activity with supposed submicroscopic cell structures. They generally did not pose questions about the significance of these structures for reproducing in an unchanged or changed form individual or sexual characteristics in succeeding generations; i.e., they did not extend their hypotheses to problems of variation, inheritance and evolution. In general, biologists who posed questions about elementary bearers of life proceeded in the main from attempts to explain, albeit approximately, the appearances of morphological and functional characteristics in successive generations, that is, they were seeking a solution of problems of inheritance and evolution.

Yves Delage, in his book "La structure du protoplasma, et les théories de l'hérédité et les grands problèmes de la biologie,"[65] gave a historical survey of speculative theories on the structure of protoplasm which, included those attempting to cover only problems of micromorphology and cell physiology and those touching on problems of individual development, variation, inheritance and evolution. In systematizing these theories, Delage first divided them into theories postulating the existence of indestructible organic particles and theories acknowledging the existence of particles which are destroyed after the death of the individual. While the latter recognized the presence of homogeneous particles, which determine various phenomena in the organism according to the particles' molecular strength, geometrical form, or their capacity for orderly movement, they also admitted the existence of various nonrepresentative and representative particles.

The representative particles were differentiated by their ability to reproduce sexual (ancestral) or individual manifestations of structure and functions present in individual cells, organs or other formations of the developed organism.

It is appropriate to begin with those hypotheses intended by their authors to form notions only about the nature of protoplasm.

Many botanists and zoologists followed Brücke in believing that supramolecular organization must exist in protoplasm. Thus W. Hofmeister in a section of "Handbuch physiologischer Botanik" entitled "Die Lehre von der Pflanzenzelle" wrote: "The substance (of protoplasm — L. B.) appears similar in all parts of the body, and it is of a liquid-viscous consistency. It is rich in water, and its particles are easily displaced relative to each other."[66] "Every attempt, "says Hofmeister in another part of the same book, "to picture the mechanism of protoplasm's mobility forces one to recognize, first of all, its organization, since in liquids or semiliquids of organized bodies molecules displace their matter equally easily in all directions... In live protoplasm displacement in certain directions outweighs movement toward other sides... The basic transparent substance of protoplasm only seems homogeneous. Only the imperfections of our present optical instruments prevent us from establishing its particular structure, which one must recognize as deriving from changes of its condition and its movement in certain currents."[67]

The attempt to consolidate notions about the structure of protoplasm led to theories, some based on empirical facts, on results of microscopic investigations and on model experiments and some of a purely speculative character. Even the empirical theories, however, were often supplemented by various speculations.

In the first group belong notions on the homogeneity of protoplasm and on its reticulate, fibrous, foamy, or grainy structure.

The presence of grains and fibers was noted in those theories which advanced notions of the homogeneity of protoplasm, but these formations were considered to be passive and changeable. Vital properties were attributed to the homogeneous semiliquid substance in which the granules and fibrillae were suspended. These views were supported mainly by botanist-cytologists, particularly by E. Strasburger, Knoll et al. Protoplasmic movement in plant cells, first described by B. Corti in 1772, was thought to be a monotonous circulation of the semiliquid cell content, which carried the granules with it. The uninterrupted intermingling of the flowing protoplasm indicated that the fibrous, reticulate, or other structures viewed in it, could not be considered real formations. A. Kölliker called the structureless basis of protoplasm hyaloplasm and the fine granules in it, microsomes. In hyaloplasm two parts were distinguished.— trophic and formative. To the first was ascribed the passive role of nutritive substance; to the latter (which Strasburger called kinetoplasm), the formation of such periodically arising and disappearing structures as radial figures, filaments, and spindles, characteristic of the processes of mitotic division.

Along with the theory of protoplasm as a homogeneous mass forming temporary structures, other theories were developed of permanent fibrous and reticulate formations in the protoplasm. After Fontana's observations (see p. 8), structures were described several times, particularly in ganglionic cells (R. Remak).[68] It was later proposed that these fibrous and reticulate formations were not a special property of the nerve cells, but were also characteristic of epithelial, particularly ciliated cells (C. J. Eberth,[69] A. F. Stuart[70]), and of the protoplasm of any cell (C. Fromann[71]).

The authors describing the fibrillar and reticulate structure of protoplasm often went beyond what was observable under the microscope in their deductions. Thus, W. Velten[72] supposed that plant cell protoplasm has a system of fine canals, with transverse partitions, filled with a watery liquid; in his opinion, the configuration of such canals is capable of changing.

Fayod's[73] notions are still more remarkable. He asserted that protoplasm and the nucleus consist of long, spirally winding fibrillae filled with a grainy plasma. He called these spinofibrillae. In Fayod's opinion, spirofibrillae cross from protoplasm into the nucleus and vice versa, and perhaps from one cell to another; even blood plasma consists of spirofibrillae. Although Fayod's descriptions of blood plasma were incorrect (he mistook fibers of fibrin for spirofibrillae) — his observations of the nucleus may have revealed again the spiral structure of the chromosome, first described ten years earlier by J. Baranezky.[74] J. Heitzmann[75] compared the structure of protoplasm to a thick net in which the granule content — a semiliquid substance — is found. Heitzmann called the fibrous reticulum the live protoplasm, but he considered the homogeneous content inert. In contrast to Heitzmann, F. Leydig called the reticulate structure spongioplasm and saw in it only a supporting framework. The only living part, in his opinion, is the homogeneous hyaloplasm. E. Schaeffer[76] later developed similar views, which he based on the fact that in the formation of pseudopodia in rhizopods only the needle-like, transparent part takes part, while the reticulate structures do not penetrate into the pseudopodia. J. Carnoy later wrote about the reticulate structure of liver cells in the monograph "La biologie cellulaire."[77] Carnoy considered the fibrous and radial formations to be only modifications of the contractile and elastic reticulate framework.

The theory of the fibrous, or fibrillar, structure of protoplasm was expressed repeatedly in the works of C. Fromann,[71] W. Schleicher,[78] and W. Flemming.[79] Schleicher, objecting to the views of Heitzmann et al. that the fibrillae in protoplasm unite into a solid net, insisted on their isolation from each other. Flemming, an extremely fine observer, referred to the fibrillar structure of protoplasm more cautiously; he noted that, while such a structure was conceivable in many cases, it is very difficult to establish, since the fine structures of protoplasm are on the border of visibility. Flemming thought the protoplasmic fibrillae may approach contiguity and even temporarily merge.

The authors of the above-mentioned, basically empirical theories of the structure of protoplasm did not connect them with any physicochemical notions about the nature of protoplasm. By contrast, O. Bütschli tried to make the structure of protoplasm conform to the laws of physics and chemistry, because, as he says in the introduction to one of his works, "... phenomena in an elementary organism become understandable only as a result of an understanding of the physicochemical conditions of their origin and destruction."[80] In this work he offers as an explanation of the phenomena of cell division, the physical properties of liquids, specifically surface tension. Bütschli later opposed to the theories of the fibrous, reticulate or grainy structure of protoplasm his own view of it as a foamy or alveolate structure. He described these views in detail in his monograph

"Untersuchungen über mikroskopische Schäume und das Protoplasma."[81] Working from microscopic observations, Bütschli attempted to model protoplasm using finely alveolate foam. He ascribed what he regarded as the error in the hypotheses of the reticulate or fibrous structure of protoplasm to a faulty interpretation of descriptions of optical cross sections of cells of foam.

L. Rhumbler, who is also known for his attempts to model the live cell, was among the mechanistic-minded biologists who supported Bütschli's views.[82] Anti-mechanist biologists also reacted sympathetically to Bütschli's theory. A. G. Gurwitsch, in interpreting the results of his classical experiments of the centrifugation of eggs, also proceeded from the notion that protoplasm has the character of foam or of an emulsion.[83] The proponents of the granular structure theory of protoplasm often criticized the foamy theory. In his dispute with Bütschli, J. Künstler opposed the vitalistic viewpoint to Bütschli's mechanistic notions, which were based on inorganic models. In his article, "Recherches sur la morphologie des flagellés," Künstler wrote, "Protoplasm is a living, highly structured substance which has nothing in common with mixtures (made by Bütschli— L. B.). It seems to me as useless to compare these two kinds of facts, from the point of view of a true understanding of the structure, as to compare the jellyfish to an umbrella, the sea-urchin to a pincushion or some pearlworts to lace."[84]

In his reply to Künstler, Bütschli counted him among those biologists who are disinclined to explain vital phenomena by physicochemical means. "A hidden curtain or mystical smoke, which still shrouds vital phenomena in one day," wrote Bütschli, "is used by them to obscure observed processes and relations with complicated allusions and false analogies to higher forms of development." They are, in Bütschli's words, the "lovers of paradoxe, who experience a holy horror before the simple solution of a problem."[85]

"As much as our knowledge permits us to judge," continued Bütschli, "plasma is structured in no more complicated way than is artificially made foam, and even if it has more complexity, it still belongs in the area of chemistry... One must not consider the comparison of a jellyfish with an umbrella as unworthy, even if the jellyfish is not made of the same substances as an umbrella from factory X. or K^0 . . .

"Plasma from the factory of nature is constructed basically the same as is artificial foam from Bütschli's factory, but it possesses the comforting advantage that the substance of its framework consists not of olive oil, but of a distinctive plasmic substance and its enchylema contains many substances which are not peculiar to foam."[86]

This exchange, so characteristic of the conflict between mechanistic and vitalistic views, shows the extreme positions adopted by both. Bütschli's mechanistic views, progressive in the sense that they set forth the cognoscibility of vital phenomena and the legitimacy and effectiveness of physicochemical investigations of these phenomena, fell into error when they were limited by superficial analogies. Künstler's position, progressive in that it contained a criticism of such analogies, became unacceptable when he declared the incognoscibility of vital phenomena.

Notions about the granular structure of protoplasm are found empirical and speculative theories merge. Various speculative propositions on the submicroscopic structure of protoplasm and of cell organoids were based on meager facts gained from study of microscopic structures, and sometimes were completely unrelated to these observations.

Künstler defended one of the first notions on the grainy structure of protoplasm. Seven years before the work cited above, Künstler asserted that the body of flagellates consists of numerous, closely adjoining hollow, protoplasmic spheres (sphérules protoplasmatiques).[87]

Other authors, particularly J. Hanstein and H. Martin were at the same time developing theories, similar to Künstler's, but more specific. Hanstein, using Kölliker's term, called the protoplasm grains "microsomes," the name by which they are still known. Martin supposed that protoplasm consists of a basic substance in which there are living granules resembling microccocci.[88]

The supporters of the granular theory speculated on the origin of the various formations they observed under the microscope. Thus, W. Pfitzner[89] thought that the active structures of protoplasm are formed from the attraction and repulsion of granules suspended in the homogeneous, basic substance of protoplasm, as a result of which there appear fibers oriented in different directions. A. Brass[90] explained the origin of the netshaped structures of protoplasm as a result of orderly grouping of the granules. F. Vejdowský,[91] in his study of the ripening, fertilization, and division of the eggs of Oligochaeta Rhynehelmis, supposed that the protoplasm of the egg is at first completely homogeneous; later, fine grains appear which group in a regular way. As a result of this, branching filaments arise and finally form a reticulate structure.

R. Altmann formulated the granular theory in a more complete form.[92] By using aniline dye, he succeeded in exposing the presence of numerous granules in protoplasm and in the nucleus. Altmann went too far, however, in interpreting the microscopic picture; he enlarged the empirical theory of the granular structure of protoplasm into a speculative theory of bioblasts (see below, p. 20).

A widespread diffusion of speculative biological theories on various aspects of the structure and vital activity of organisms characterized the last decade of the 19th century. Speculation was unavoidable on the part of biologists with a vitalistic viewpoint, since a vitalistic construction can only be conceived by introducing a fictitious principle into organic nature. Biologists of a materialistic outlook who advocated a position of mechanistic materialism which would utilize current methodology were also often forced to hypothesize to fill in the gaps of empirical research. At the basis of most speculative theories which were used to interpret the structure of the material basis of vital phenomena and the regularity of individual development and heredity lies the corpuscular principle, i.e., the notion that the material basis of life is discrete and consists of individualized structural units.

Buffon's theory — (mentioned above) — of "living molecules," "indestructible particles" was one of the earliest of these. Some 130 years later A. Béchamp described a similar notion — his microzyma theory — in a work of more than 1,000 pages.[93] In Béchamp's opinion, organized bodies consist of microzymas, round particles, created by God, immortal and dispersed everywhere. Microzymas unite for the formation of organisms, and disperse after the death of the individual. Béchamp ascribed to microzymas the ability to produce enzymes which cause fermentation in the appropriate substrata. Béchamp thought that new cells rarely occur through multiplication, but rather that they are usually formed from uniting corpuscles — microzymas. Afterwards, this aggregate secretes a membrane. on the surface. He attributed similar attributes to the cellular element of blood and to sexual cells. In discussing Béchamp's views, Yves Delage remarks that these theories cannot explain the transmission of traits from parents to children or the specific structure of cells — how "from one and the same elements are formed now the leaf of a plant, now the mollusk's covering, now the liver, brain or the kidneys of mammals... There is no explanation of how so many different formations are developed from microzymas. Page follows page without answering objections to the theory. It is boring and useless to read all this."[94] Béchamp's reversion to theories already discredited 150 years earlier is indeed a remarkable relapse, occurring, as it did, at the end of a century of inventiveness in natural philosophy.

Foremost among those speculative theories which assume that chemically identical particles from the composition of organisms and account for the great variety of the organism's properties, are Spencer's theory of physiological units, Haake's theory of gemmaria and the Haeckel-Erlsberg theory of plastidules.

H. Spencer considered the organization of cells too complicated to explain by a simple combination of chemical elements. In Spencer's words, "... the units of which any organism is built have an innate tendency to arrange themselves in the shape of that organism... A plant or animal of any variety is composed of special units which have the ability to be aggregated in the form of this species." And further on, "Life-giving particles, forming tissues, show the tendency toward a particular grouping — whether this tendency is revealed in the reproduction of the whole form or in the reinforcement of its imperfections."[95] Elementary vital properties belong, in Spencer's opinion, neither to chemical nor to morphological units, i.e., neither to atoms, nor to molecules, nor to cells. Proceeding from there, Spencer posited the existence of molecular units of an intermediate order between chemical units (atoms and molecules) and morphological ones (cells). These hypothetical physiological particles postulated by Spencer, which he considered to be the smallest particles of live matter, significantly anticipated later biochemical notions.

According to Spencer, the diversity of physiological units depends, not on the differences in the chemical composition or quantity of component molecules, but on the dissimilar interdependent positioning of molecules in space. The instability of a physiological unit in relation to external influences increases directly with its complexity. Spencer illustrated this by the following examples: lead melts as 328°C, tin at 293°C, bismuth at 325°C, while their alloy melts in boiling water. Spencer tried to apply the theory of physiological units to phenomena of individual development, regeneration, asexual and sexual reproduction, to problems of taxonomy and of heredity.

W. Haake's theory was considerably more schematic.[96] In his opinion, protoplasm consists of uniform elementary particles (gemmae), comparable to crystals, which, by mutual attraction, form structures of a higher order (gemmaria) of a size which is beyond the resolving capacity of the microscope.

The concept of an aggregate - atomic or molecular - composition of protoplasm lies at the basis of theories formulated by Haeckel and later worked on by Erlsberg. E. Haeckel[97] gave the name of plastidules to similar hypothetical aggregates of atoms, i.e., very complex molecules lying beyond the limits of visibility. They maintain their vital properties until they break down into their component atoms. In Haeckel's opinion, plastidules do not reproduce by division, but arise in a nutritive liquid, just as new crystals appear from a crystal placed in a saturated solution. Haeckel tried to explain reproduction, heredity, adaptation and evolution of organisms by the activity of plastidules. In the book cited above, Yves Delage replied sharply to Haeckel's views. In Delage's opinion, Haeckel's theory and his mechanistic explanation of vital phenomena, contain "revolting metaphysical rubbish (exécrable fatras metaphysique), unworthy of a naturalist of this (19th – L. B.) century." "Haeckel," wrote Delage further on, "tries to create before the reader's eyes a veritable phantasmagoria of invented effects (fait papilloter devant les yeux du lecteur toute une phantasmagorie)" in which he ascribed sensitivity, and even will and memory to plastidules. Haeckel's tendency toward an electric mixture of mechanistic and idealistic positions is well known and it is not surprising that his theories drew such a heated reproof.

L. Erlsberg formulated a variant of the plastidule theory[99]: protoplasm of cells consists of units which form the genuine physical basis of life. These units neither grow nor are reproduced, but their unbroken continuity is insured by the appearance of new units among and under the influence of old units, which are, in this way, replaced as they die. Erlsberg's theory of plastidules, like Haeckel's, pretends to explain all vital phenomena, free, however, of that touch of hylozoism which is present in Haeckel's constructions. Erlsberg's theories, hypothetical and speculative as they are, contain a prefiguration of much later theories – some speculative, some empirical – on the continuity of embryonic plasma (Jäger, Nussbaum and Weisman) and on the reproduction of molecular structures according to a previously existing pattern (Chevreul).

The notion that protoplasm might be composed of molecules of a dissimilar chemical nature represented a certain advance. J. Hanstein's and G. Berthold's distinctly vitalistic views of protoplasm as an accumulation of protein molecules, Altmann's theory of bioblasts and Weisner's theory of plasomes all stemmed from this notion.

Hanstein called the protein substance plastin, and postulated that molecules of inorganic substances (oxygen, carbon dioxide, phosphorus, sulfur, etc.) move between the plastin molecules. The molecular complexes of plastin are modeled, in Hanstein's opinion, by unknown forces of the molecules themselves, so that the organized form and structure depend upon the nature of the molecules. Hanstein asserted that the physicochemical processes in protoplasm are subordinated to the general forming and supporting plan of vital activity (Gestaltungs- and Erhaltungsplan). "They depend," wrote Hanstein, "upon the will and demands of the individual spirit for whom they are the substance and means."[100]

G. Berthold's notions on the nature of protoplasm also had a vitalistic character. Berthold's views, based on the principle of dynamic teleology, can be characterized by his own words: "The essence of an organism, which differs so noticeably from any mechanistic system, is not found in any special molecular structure, so that it would be utopian to try to penetrate into the intimate phenomena of life by this means. The live substrata of organisms, protoplasm, does not depend in its properties on any physicochemical peculiarities of any single substance, such as protein, for example (although protein is found in all protoplasm, and without it protoplasm cannot be formed); nor are its properties dependent upon its structure or organization, which is not different than that of any emulsion. A circulation of forces and substances characterizes live protoplasm... It is important that, by means of its own chemical energy, ... it maintains itself, grows, and multiplies, that, by the action of this energy, forces are ceaselessly released which, at any given moment, are such that they correspond to the needs of the organism to create its own particular form and to accomplish necessary movements."[101]

While Hanstein's and Berthold's views have an abstract, hypothetical character, Altmann's theory of bioblasts mentioned above (p. 17), derived from empirical observations.[102] Altmann connected the granules of protoplasm seen under the microscope with specific functions of cells, in particular of fat and gland cells. Expanding the notion of the grainy structure of protoplasm beyond the visually perceived limits, Altmann proposed that the fibrillar and reticulate structures, as well as the homogeneous appearing protoplasm, are built from tiny granule-bioblasts, which, in his opinion, are the elementary units of life. Altmann thought that granules, like cells, are reproduced by division; he therefore paraphrased Virchow's statement, "every cell — from a cell" with the aphorism, "every granule — from a granule." In discussing the nature of bioblasts, Altmann likened them to crystals which differ, however, from inorganic crystals in the way in which they grow: inorganic crystals grow as a result of the accumulation of matter from a mother-liquor on their surface (juxtaposition), while bioblasts are fed by the penetration of substances within the bioblast (intussusception). Altmann projected Virchow's idea of the microscopically visible colony or kingdom of cells within the organism to visually imperceptible granules of protoplasm and conceptualized the cell as a colony of bioblasts.

The botanist, J. Weisner,[103] developed similar theories; he called the elementary particles of protoplasm plasmatosomes (or, in short, plasomes). Weisner generalized erroneously when he applied the principle of reproduction by division (as in the case of the cell as a whole and its components — centrosomes, nucleus, chromosomes and perhaps others) to hypothetical submicroscopic units of protoplasm.

M. Heidenhain, like Weisner, recognized divisibility as the most important characteristic of organized bodies and he developed Haeckel's idea on the graduated succession (Stufenfolge) of morphological systems. Heidenhain used the term, biosystem, for an aggregate of morphological formations of an organism which can be divided into biosystems of a lower order. To a certain extent this picture corresponds to actual observations: an organ (for example, a gland) can be divided into lobules and the latter into cells; the cell is also capable of division in some of its parts, such as the nucleus and controsome. In order to establish a limit of divisibility of live matter under biosystems, Heidenhain assumed the existence of elementary units. He called them protomeres and ascribed to them the capacity for autonomous metabolism, growth, and reproduction. "One must make clear," wrote Heidenhain in his work, "Plasma and the Cell," "when these judgments leave empirical ground and enter into the realm of theory. In the transition from experiment to theory, a judgment is made that: given the fact of an undifferentiated mass, as described from microscopic observations, and given the ability of organized bodies to divide — therefore, the mass consists of an aggregate of minute individualities. The existence of a consecutive order of divisible bodies provides a sufficient basis for such a deduction, which is not a jump in the dark."[104] Clearly, however, any transition from real subdivisions of an organism to hypothetically postulated submicroscopic units of structure will inevitably be "a jump in the dark." In this sense Heidenhain's "protomeres" are no different than "physiological units," "plastidules," "plasomes," or other fictional, elementary vital structures.

5. THEORIES OF REPRESENTATIVE PARTICLES

Theories based on the assumption of the existence of representative particles have a special place among speculative attempts to develop an idea of the substrata of vital phenomena. Despite their clearly hypothetical character and the contentions they often aroused, they were at one time widely held.

These theories emerged as an attempt to explain the phenomena of heredity, i.e., the resemblance between successive generations of living beings materially connected with each other. It was thought for a long time, that heredity traits were transmitted by a shapeless substance which was detached from the maternal and paternal organisms. A discrete structure responsible for this transmission was ascribed to this substance and, later (when ovules and spermatozoa were discovered), to the substance of these sexual elements.

The idea that the origin of the "substance of heredity" lay in all parts of the organism of both parents arose in very distant times. One can trace this idea through history to the opinions of philosophers of ancient times, when it was already a subject of discussion. Aristotle wrote: "Now it is thought that all animals are generated out of semen and that the semen comes from the parents." "Some say that it comes from the whole of the body," he continued. Advocates of this view, in Aristotle's words, refer to the resemblance of children to parents, which is distributed over separate parts of the body ("if then the coming of the semen from the whole body is cause of the resemblance of the whole, so the parts would be like because it comes from each of the part"). They also speak of the repetition of injuries and deformities in posterity ("for they argue that since the parent is deficient in this part, the semen does not come from thence, and the result is that the corresponding part is not formed in the offspring").[105] By these arguments they tried to explain that "children are born with a likeness to their parents not only in congenital characteristics, but also in acquired traits." Aristotle thought, however, that, "On examining the question, however, the opposite appears more likely, for it is not hard to refute such arguments... First, then the resemblance of children to parents is no proof that the semen comes from the whole body, because the resemblance is found also in voice, nails, hair, and way of moving, from which nothing comes. And men generate before they yet have certain characters, such as a beard or grey hair. Further, children are like their more remote ancestors from whom nothing has come, for the resemblances recur at an interval of many generations... The same thing applies also to plants, for it is clear that if this theory were true the seed would come from all parts of plants also; but often a plant does not possess one part, and another part may be removed and a third grow afterwards."[106]

And in another place: "Yet again if the whole animal or plant is formed from semen or seed, it is impossible that any part of it should exist ready made in the semen or seed..."[107]

C. Darwin constructed his theory of pangenesis on exactly those arguments against which Aristotle had so convincingly argued, and named Heraclitus and Hippocrates as his predecessors. He would have had greater reason to refer to Democritus and Paracelsus, whose arguments were no more conclusive than those which Aristotle disputed. Apparently, the more immediate predecessors who influenced Darwin in the formulation of his unsuccessful hypothesis were Bonnet, Maupertuis, E. Darwin and Spencer.

In the beginning of the 19th century, Hösch,[108] repeating the suppositions of Maupertuis and E. Darwin (see p. 8) pictured hereditary matter as a mixture of substances or of embryos of all organs, conveyed to the testicles and ovaries by a flow of lymph and blood.

G. Spencer, speaking about the hierarchical structure of living matter (atoms or molecules, hypothetical physiological units, morphological units, i.e., cells) attributed to physiological units the ability to form morphological structures of a developing organism that corresponded to parental traits. An aggregate of representative physiological units in the sexual cells of father and mother fulfills this role. In Spencer's opinion, they are also present in the other body cells. Spencer's hypothesis, therefore, did not need a supplementary assumption of the transfer of representative units from the cells of the body to reproductive cells.

Darwin's theory of pangenesis rests on notions on the existence of submicroscopic embryos, gemmules, which are able to penetrate membranes and thus go from one cell to another. Darwin, like Hösch, assumed that gemmules are transmitted from cells of the body through circulation systems to the sexual cells. Darwin himself regarded the hypothesis of pangenesis as a provisional solution and he was not satisfied with it.[109]

The arguments of W. Brooks in his book, "The Law of Heredity"[110] represent an insignificant modification of the hypothesis of pangenesis, even more arbitrary than Darwin's. Brooks attributed gemmules only to spermatozoa. While the gemmules, in Brooks' opinion, change during the life of the father, the ovum contains unchangeable germs, representatives of all parts of the maternal organism. His assumption about the invariability of the ova's germs led Brooks to the incorrect assertion that hereditary variations depend only upon the father.[111]

In 1886 P. Hallez published the brochure "Pourquoi nous ressemblons à nos parents?"[112] In it he proposed another speculative hypothesis of heredity according to which the determinants of hereditary characteristics are contained in the cytoplasm, while the nucleus serves as the cell center attracting particles of cytoplasm, which prevents the cells from merging into syneytium. On the basis of erroneously interpreted observations of protozoan division, Hallez concluded that cytoplasmic gemmules, micelles, plastidules, and even molecules of organic substances are characterized by an unstable position in space. Constant positioning of cell components is maintained even after division; this also applies to sexual cells, which reflect the stereometrical structure of the cells of the body which engendered them. Proceeding from a false assumption that the components of the cell turn into whole cells and later into organs, Hallez thought that the orientation of molecules of cytoplasm predetermines the placement of cells in the organ and of organs in the organism. During the splitting of the ovum and the division of cells of the developing embryo, the rudiments are separated into definite groups, which grow continually finer and more specialized. The assortment of heredity rudiments is present a priori; the splitting of the ovum and division of cells only subsequently creates barriers between blastomeres, embryonic blades, rudiments of organs and, finally, between completely formed cells of the grown organism.

Gaule's "theory of cytozoen"[113] which suggests that cells consist of various combinations of tiny living beings, has a completely fantastic character. These beings, called cytozoen, are released after the death of the cell and initiate new cell growth. They are carried in the bloodstream into sexual cells, which, as a result, acquire the ability to reproduce traits of the entire parental organism. An erroneous observation is at the empirical basis of this theory: Gaule mistook blood parasites of the frog for component parts of blood cells and enlarged this observation to all cells of multicellular animals and plants.

Other hypotheses attempting to explain the transmission of hereditary traits developed ideas about material bearers of heredity and about their participation in the development of form. The views expressed on this often went far beyond the limits of the originally posited problem and claimed to interpret not only characteristic individual development and heredity, but also evolution. Among such is Naegeli's micellar hypothesis, set forth in his book, "Mechanisch-physiologische Theorie der Abstammungslehre."[114] Naegeli proceeded from physicochemical investigations, in particular from data of colloidal chemistry. Basing his ideas on organized matter's ability to swell, on the insolubility of this matter in water, and on its capacity for dual refraction, Naegeli proposed that protoplasm consists of micelles, elongated combinations of protein molecules surrounded by a layer of water. Protoplasm, according to Naegeli, is made up of two kinds of micelles: unordered ones which serve as a nutritive material, and those in parallel arrangements. which form embryonic plasma, or idioplasm. A net of micelles of idioplasm permeates the entire body, including the sexual cells, and causes the development of specific and individual traits of the organism by molecular action of these micelles. Like similar hypotheses, Naegeli's depended upon unobserved cellular structures, which, even in the case of the very important organic molecules forming micelles, Naegeli thought to be beyond the limits of the microscope's resolving power.

Histologists viewed Naegeli's opinions as insubstantiated and improbable. A. Kölliker[115] criticized his theory of the penetration of a micellar net of idioplasm into all cells of the organism. Kölliker assumed that micelles of idioplasm could be concentrated only in the nuclei of cells, forming there nucleinic nets. Neither Naegeli nor Kölliker explained how micelles or groups of micelles of idioplasm provide for the organization of living bodies.

Attempts to connect the discrete structure of the developed multicellular organism with the discreteness of hereditary structures in sexual cells led to the creation of new hypotheses of representative living bodies.

At the basis of these hypotheses were observations from genetic and cytological investigations: the equal hereditary potentials of paternal and maternal organisms; the similarity in size and structure between the nucleus of the ovum and the head of the spermatozoon; and the exact distribution of nuclear structures in cell mitosis particularly among daughter cells.

Hertwig remarked that Naegeli's speculative theory of idioplasm assumes the inequality of female and male sexual cells, but "leaves as open questions which substance in sexual cells is idioplasm and which is the ordinary nutritive plasma," and, also, "whether it is possible to discern them under the microscope."[116] After O. Hertwig[117] et. al. elucidated the concept of amphimixis (i.e., the mingling of paternal and maternal characteristics by union of male and female pronuclei in fertilization) he (Hertwig)[118] and E. Strasburger[119] simultaneously proposed that the nuclei of sexual cells should be considered the bearers of hereditary dispositions, and their chromatin as the substance corresponding to Naegeli's idioplasm.

At the end of the 19th century, Weismann's was the most elaborated of the speculative theories of the structure of the substance which assures regularity of development and of heredity although his basic idea was expressed earlier by Jäger, Rauber, and Nussbaum.

G. Jäger wrote in "Handbook of General Zoology" and later in a special article[120] that the organism of higher animals is made up of two kinds of cells, "ontogenetical" and "philogenetical." The latter, which take part in reproduction, are not the product of the former, but originate directly from the parent egg cell. He thought that the formation of the "reproductive substance" occurs during the first stages of embryonic development of animals. A. Rauber, Professor of Anatomy of the Yurev (now Tartu) University expressed a similar thought in the article, "Formbildung und Formstörung in der Entwicklung von Wirbeltiere" (Formation and Destruction of Form in the process of Vertebrate Development), in which he wrote: "In fertilization only one part of the egg contributes to the form of the individual, its individual part, but the other part does not exert this influence." M. Nussbaum perceived that "the splitting egg divides into cellular material of the individual and into cells designated for the maintenance of the species."[121]

A. Weismann also differentiated two plasmas in the organisms — the individual or somatic, taking part in the creation of the body of the individual, and the embryonic, characterized by specific continuity, i.e., the transmission of sexual cells of one generation to the sexual cells of another. Weismann attributed a complex structure to the embryonic plasma. Its basic units, biophores (bearers of life), are a grouping of molecules of organic matter. Biophores are found in the cell nucleus, through the "pores" of which they are able to move out into the cytoplasm or morphoplasm, a passive live substance formed under the influence of biophores. Biophores of embryonic plasma merge into regularly constructed groups, determinants, upon which the structure of each cell depends; consequently, there should be as many determinants in embryonic plasma as there are heterogeneous cells. In the case of homogeneous cells, one initial determinant is sufficient to propagate itself in the quantity necessary for the preservation of all similar cells. Determinants in turn are ordered in the embryonic plasma of the nucleus; they are grouped into units of a still higher order, ids, each of which contains all the determinants necessary for the development of the whole organism. Ids, like biophores and determinants, are nourished, grow and propagate themselves by division. At definite moments of the cell's life, namely at the time of its mitotic division, ids merge into idants, which correspond to the microscopically visible nuclear structures — chromosomes.

A variation of Weismann's theory is H. de Vries' theory of intracellular pangenesis.[122] De Vries postulated pangens that are microscopically imperceptible, organic particles consisting of a large number of chemical molecules. The composites (pangens) are differentiated from their components (molecules) by the capacity for nutrition, growth and propagation by division. Each cell has a large quantity of active pangens, which dtermine all structural and functional properties of separate cells. Although the pangens of protoplasm characteristic only of that kind of cell, the nucleus, in de Vries' opinion, contains all the kinds of pangens characteristic of individuals of a given species. Nuclear pangens, connected with chromatin, are in an inactive condition and, in this form, are transmitted from the maternal cell to the daughter cells during division, in which process, the pangens too are divided. In crossing from the nucleus into protoplasm, pangens are propagated and activated. By this intracellular selective migration of pangens, de Vries tried to explain the processes of cell differentiation and the formation of tissues by cells.

O. Hertwig's theory is little different from de Vries' "intracellular pangenesis." Hertwig spoke very cautiously about the realization of traits in ontogenesis under the influence of particles of heredity substance of the nucleus (idioblasts or bioblasts): "As much as it seems... proper to seek... the unknown cause (of realization of hereditary traits — L. B.) in the material property of a hereditary mass, so much would it be arbitrary and erroneous to think that the cause is to be found only in the presence of a definite material particle, a particular bioblast, determinant, biophore, etc. With the same degree of probability it can consist of either a particular position of the bioblast in a system of others, or in a particular combination of two or more bioblasts in a closer complex — or, to use general terms, it can lie in a configuration of a material system or its separate component parts."[123]

The simplified concepts of the "substance of heredity" held, for instance, by Hertwig, Weismann, and de Vries evoked a reaction on the part of physiologists and embryologists. Thus M. Verworn declared that the "physiological mode of thought cannot be reconciled with the notion of a single hereditary substance which is localized somewhere in the cell and is transmitted through propagation." Verworn thought that the traits of the cell are determined by its distinctive metabolism. The inheritance of traits of a given cell means the inheritance of its characteristic metabolism; consequently, the daughter cell must receive its somatic relationship between nucleus and cytoplasm from the maternal cell. The physiological principle to which Verworn tried to subordinate phenomena of inheritance and formative processes in ontogenesis had a purely mechanistic basis. Verwon thought that morphology, like physiology, ought to become the physics and chemistry of organisms. "Certain important morphologies," he wrote, "are captive to dogmas which must disappear with a deeper penetration (into the essence of vital phenomena — L. B.), but, meanwhile, they make up that remnant of mysticism which favors vitalistic interpretations."[124]

E. Conclin spoke out even more energetically against the connection of hereditary potentials with the nucleus. He wrote: "Since heredity includes a series of vital processes, such as assimilation, growth, division and differentiation, it is naive to think that these processes are localized in one special part of the cell. This recalls doctrines of times long past when life was thought to be localized in the heart or blood and the soul was thought to reside in the epiphysis or in the ventricles of the brain."[125]

Similarly skeptical voices have been raised over the years that have intervened until now, when discoveries of the hereditary role played by DNA and RNA have somewhat stilled them. With these discoveries, a real perspective was opened which connects the doctrine of the localization of the material (chemical) bases of heredity in the nucleus with the chemical individuality of cell proteins, which are responsible for its structural and functional characteristics.[126]

6. FROM SPECULATIVE CONSTRUCTIONS TO DEFINITIVE STUDY OF PHYSICOCHEMICAL BASES OF VITAL PHENOMENA

Toward the end of the 19th century, interest fell off in hypothetical constructions on the nature of the material basis of life. Voices were more frequently heard which called for a change from speculative hypotheses to more detailed and exact investigations of the structural and functional properties of the cell — of cytoplasm, its organoids and the nucleus. Yves Delage, in his book which has been frequently mentioned here, recommended restraint in expressing speculative judgments on the nature of protoplasm if the details of the discussed phenomena cannot be argued reliably from established facts. In general, such hypotheses, in Delage's opinion, ought to be built by the inductive method and rest on data of observations and experiments.

Live protoplasm, according to Delage's notions, should be regarded as a very complex chemical substance, constructed basically of heterogeneous proteins, partly mixed together, and partly positioned in a definite manner. The loss of vital properties of protoplasm as a result of the mechanical destruction of its structure corroborates this statement. Delage correctly noted that aggregates of chemical substances of protoplasm cannot be constant structures (plasomes, micelles) since a current of substances continually goes through protoplasm and reacts with its component parts. The disturbed condition of protoplasm is continually restored. This continual destruction and restoration is interrupted only temporarily with the destruction of protoplasm. At any given moment parts of protoplasm are differentiated from each other. But this does not require the assumption of permanent individualized elementary units of structure. Some structural components of the cell are distinguished by remarkably stable reproductivity; chromosomes, belong in this category. Delage wrote, "We must accept this conclusion without inquiring whether it is suitable or restrictive for our theory."[127] Delage thought that, beyond doubt, chromosomes are not identical to each other and that they are heterogeneous in length, i.e., the nucleic microsomes forming them are different. Otherwise, there would be no necessity for their lengthwise fission during cell division, and this complex process would be eliminated from the life cycle of the cell as useless.

Delage thought that the phenomena of diffusion and in particular of osmosis are very significant in the functioning of the cell as a whole and in the initiation of its separate structural formations. The microscopically viewed membrane can be detected on the surface of the cell and around the nucleus. There is reason to consider, however, that formations functioning as semipermeable membranes exist in other parts of the cells, as well. Delage alluded to the observations made by de Vries[128] which affirmed the existence of a membrane around vacuoli and to Boveri's investigations[129] which showed that archiplasm, or the centrosphere, although without a visible membrane, acts as if it had one. It is difficult to understand how the integrity of these formations is preserved in the face of various interferences unless one assumes that the surface of the astrosphere is differentiated by a coating which does not interfere with the remaining cytoplasm. "It would not be unscientific speculation to suppose," wrote Delage, "that any individualized part of the cell is limited by a membrane, constructed in the nature of a somewhat thicker peripheral layer which is differentiated when in contact with the surrounding milieu... This hypothesis can be rejected if one wishes to remain within the borders of exact observation; it is, however, worth the recognition that it is not improbable, nor without foundation — that it is legitimate and not too audacious."[130]

At the end of the 19th and in the beginning of the 20th century, the successes of chemistry, particularly in studies of metabolism and of the physiological role of enzymes were bound to affect cytology, which, until then, had worked chiefly by morphological methods. Biochemistry, however, in turning to intracellular processes, met with great methodological and theoretical difficulties.

Franz Hofmeister's well-reasoned brochure, "Die Chemische Organisation der Zelle," played an important historical role in opening new perspectives on the biochemistry of the cell.

Hofmeister thought it necessary first of all, to counter a rebuke often thrown at chemists, that it is no more possible to explain vital manifestations of protoplasm by chemical investigations of lifeless protoplasm than it is possible to study the mechanism of broken watches by chemical analysis. Hofmeister considered this reproof not fully justified, and in some respects simply false, since substances can be extracted from the destroyed cells which display in the test tube an activity close, if not identical, to that which is characteristic of it in the organism; this applies primarily to enzymes. The biochemist cannot explain the microscopically viewed structure of protoplasm; but he proposes to reveal much smaller structures lying far beyond the limits of visibility — a spatial configuration of molecules of organic substances. The judgements of cytologists on the functional significance of separate cell structures were reasonably well substantiated when established formations were under discussion, such as the nucleus and grains of chlorophyll and starch. In the case of smaller structures, however, such as granules and protoplasm, and (even more so) structures past the range of vision, their speculations were, in Hofmeister's words, rather "more bold than fruitful..."

Hofmeister posed another problem: "to proceed, not from the visible structure of the cell or of protoplasm, but from their activity, and, thus to explain how the cell and protoplasm must be constructed in order that this activity be possible."[131]

Hofmeister began his studies with liver cells, which simultaneously fulfill several independent functions. The multi-functionality of liver cells would indicate to the biochemist a spatial division of some kind for the realization of all these functions (just as in a laboratory various vessels exist for different chemical processes). The structure of the liver cells, however, shows nothing of this division of labor; they are all similar in their structure and in the distribution of blood and lymph vessels and bile ducts. It is, therefore, impossible to think that one cell is occupied with the consumption of glycogen, another with its synthesis, a third with the formation of urea, and a fourth with the secretion of bile. "In the liver cell," wrote Hofmeister, "the microscope reveals an empty scene, and only under specific conditions does it successfully create separate, visible episodes in an invisible theatrical performance, for example, the accumulation of glycogen in paraplasm or the formation of secretory vacuoli."[132] One can resolve these questions, in Hofmeister's opinion, by conceptualizing "the chemical organization of the cell"[133] which should no longer be compared with the organization of a machine.

Hofmeister schematized phenomena which were studied in detail much later, but which were practically unknown in the beginning of the 20th century . He assumed the presence of some ferments in the fertilized egg whose activity produces new chemical combinations, among them new ferments of pro-ferments, "until chains of the new chemical formations required by the history of the species are realized. Epigenesis of form is therefore an expression of epigenesis of chemical forces... Here, unless one prefers transcendental explanations, lies a key to simultaneous realization of morphological differentiation."[134]

A basic question of intracellular processes, remains as to what mechanism preserves the spatially inviolable course of vital reactions. "What is the cell as a whole — a vessel filled with a homogeneous solution, in which all chemical processes take place, or many separate vessels for the uninterrupted course of separate reactions occurring simultaneously or in regular succession?"[135]

Cell pro-ferments or ferments are incapable of diffusion; they are, therefore, fixed to that part of the protoplasm in which they were formed and are activated only by an appropriate chemical substance. "This concept requires that there be many colloidal partititons in protoplasm — a conceivable notion to anyone who knows the tendency of many colloidal bodies to readily form membranes on all of its surfaces which come in contact with the surrounding milieu."[136] Without the presence of the colloidal membranes to form small, separate sections of protoplasm, "it is difficult to understand how variegated, and often opposing, processes — hydration and dehydration, oxidation and reduction — can occur alongside each other in protoplasm without disturbance... A regular succession of chemical reactions in the cell supposes the separate action of chemical agents and a definite direction of movement by the formed products, in short, a chemical organization."[137] Hofmeister postulated a spatial disconnection of chemical processes and assumed the existence in protoplasm of semipermeable cells, which, although submicroscopic, are essential for these processes.

Hofmeister distinctly saw the basic characteristic of vital processes as unity of structure and function. The brochure's concluding words, which testify to his materialist views, are worth quoting in full.

"If the morphologist," he wrote, "attempts to explain the structure of protoplasm in the smallest detail, and the chemist on his part, attempts to explain by coarse but deeply penetrating means the chemical activity of protoplasm with his methods (which, however coarse they appear, are actually deeply penetrating) – then the concern of both is for two sides of the same thing. The final goal for one is as detailed a description of the structure of protoplasm as is possible, and for the other, it is the description in chemical and physical terms of all the processes which take place in the protoplasm. The relationship between these two widely differing concepts is a difficult, but promising problem for the future. One can already say, however, that the concept of the cell as a machine operating by chemical and physicochemical means will at no time lead to the supposition of any but known forces, and that, as far as can be foreseen, there is no cause for that discouragement which, on the one hand, admits 'ignorabimus,' and on the other, resorts to vitalistic conclusions."[138]

R. Höber, in "Physikalische Chemie der Zelle und der Gewebe" (The Physical Chemistry of the Cell and Tissues),[139] which has been frequently reprinted, attached great significance to Hofmeister's views. He noted in addition that protoplasmic structures have the character of temporary formations, particularly in the cases of pseudopodia, infusoria and some plant cells where the movement of protoplasm is so fast that colloidal cells cannot be fixed structures. The experiments of A.G. Gurwitsch confirmed the unstable character of intracellular formations.[140] This author showed that after an energetic centrifuging of the eggs of sea-urchins and amphibians, the yolk was sharply dislocated and the fine structure of the protoplasm was disturbed, but the vital activity was not essentially disturbed since the centrifuged egg retained the ability to split. The juxtaposition of these facts indicates that protoplasm lacks a rigid frame as postulated by many authors who studied the cytological consequences of centrifuging. None of them, however, had carried this assumption through to its conclusion. E. Wilson, expressing himself most cautiously on this theme, considered that "it is difficult to imagine an intercellular substance that is sufficiently firm to support its typical configuration and, at the same time, sufficiently plastic not to obstruct the wide movement of granules and other content through it."[141]

O. Warburg's work, "Uber die Wirkung der Struktur auf chemische Vorgänge in Zellen" (About the Influence of Structure on Chemical Processes in Cells),[142] is concerned with the connection between biochemical transformations in cells and their internal structure. Warburg noted that many fermentative reactions can take place outside the organism, for example, the action of digestive enzymes. The biochemical processes which coexist with them seem unconnected with cell structures, but this independence is entirely relative. For example, the action of yeast cells (the conversion of sucrose into glucose) can be halted by cultivating yeast in a solution of glucose without disturbing their growth and propagation. However, it is impossible to consider fermentation of yeast as completely independent of the structure of yeast cells since yeast juice shows a significantly lower intensity of fermentation than a corresponding quantity of intact yeast. Warburg thought that the fermentative activity depends, not on the molecules' properties, but on the protoplasmic formations of a higher level, i.e., on the micromorphological structures of cell protoplasm.

Moreover, various biochemical phenomena, in particular, reactions involving the liberation of energy, are undoubtedly directly connected with the vital activity of cells. The rate of such reactions is directly related to the intensity of vital processes and with the rate of growth and of cell division. The process of oxygenation in breathing is an example.

Initially it was assumed that cellular oxygenation is localized in the nucleus.[143] Warburg considered this unfounded since it is known that the axial cylinder of the nerve continues to respire when separate from the ganglion cell – the movement of protoplasm in anuclear fragments of cells ceases without access to oxygen — anuclear erythrocytes of mammals respire and in nuclear erythrocytes, the intensity of respiration increases as the intensity of the staining of their protoplasm is increased, i.e., the more the protoplasm contains insoluble structural components. These facts only indicate, however, a parallelism between the mass of structural formations and the intensity of respiration, but do not indicate a direct dependence of the respiratory function upon intracellular structure. An experimental proof of this dependence is found in a comparison of the intensity of respiration in undamaged cells and in cells subjected to cytolysis, centrifuging and grinding with sand.

The necessary condition for the realization of various metabolic phenomena in cells is that the reacting substances be dissolved in protoplasm." The old statement: 'Corpora non agunt nisi soluta' is nowhere as meaningful as it is in physiology," wrote Höber.[144] It is entirely natural, therefore, that the relationship of protoplasm and cells, as a whole, to water and substances dissolved in it should long ago have attracted investigators' attention. The study of the phenomena of osmosis, i.e., diffusion through semipermeable membranes, played a large part in solving problems of cell physiology. Dutrochet initiated these investigations with the construction of an instrument to measure the osmotic pressure with the aid of an animal membrane.[145] Later M. Traube created artificial, semipermeable, "precipitated" membranes of copper ferrocyanide films.[146] W. Pfeffer, using Dutrochet's idea, constructed an osmometer in which Traube's precipitating membranes were used.[147] With his models, Pfeffer tried to imitate the phenomena of turgor of plant cells, and H. de Vries showed the possibility of determining osmotic pressure of solutions in live cells with the aid of plasmolysis.[148]

The work of Pfeffer and de Vries has a noticeable influence on physical chemistry since it established important natural laws from which the theory of solutions was derived. J. van't Hoff formulated a general theory which S. Arrhenius defined more precisely for solutions in which molecules of the dissolved substance are dissociated into ions. The study of electrolytic dissociation led to the determination of ion concentrations in solutions, in particular of H and OH ions, a type of reaction which plays such an important role in vital phenomena.

Further study of physicochemical properties of protoplasm, cell organoids, cell membranes and other surface formations led to the posing of new problems and to the setting apart of a wide field of investigations of vital phenomena which at present is often called molecular biology. This appellation is unfortunate because an indiscriminate use of the term "molecular biology" can produce a new division of biology into physics and chemistry. It is essential, however, for all the disciplines involved that biological, physical and chemical research proceed cojointly, since this type of interdisciplinary investigation can explain for the biologist the physicochemical bases of vital phenomena and can broaden the physical scientist's scope in applying physical and chemical laws.

In natural heterogeneous systems (particularly cells), and in model heterogeneous systems artificially created to simulate the natural, it is possible to study those significant phenomena which are characteristic of the statics and dynamics of the systems: surface phenomena contiguous to the external milieu and between different phases within the system (permeability, surface tension, border potential, etc.) and physiochemical phenomena related to the colloidal nature of protoplasm and its derivatives (the properties of sols and gels, coagulation, swelling, viscosity, etc.).

A special area of the chemistry of protoplasm touches on a problem equally relevant to cytophysiology, submicroscopic morphology and genetics — namely, the problem of autoreproduction of organic structures.

The historic development of concepts of the material basis of vital phenomena cannot be separated from the evolution of theories on the essence of life. The basic problems are the chemical nature of the substance of which living bodies are made, the form and structure of these bodies and their vital activity, i.e., the aggregate of their functional features which has often been fragmented at various stages in the history of biology. In some instances basic vital characteristics are principally or entirely connected with substance, in other cases, with form or structure, or in still others, with function.

The reduction of the essence of living bodies to the matter forming them goes back to ancient notions antedating science. The primary element — for Phales, water; for Heraclites, fire — becomes, according to them, the source of all matter, and questions on the source of this transformation and on the further development of form in natural bodies, including living ones, were not yet posed at that time. They were, however, soon given consideration. Empedocles, saw principles forming matter that are beyond the four material elements (fire, air, water and earth); according to him, there are two such principles: a uniting one — love, and a dividing one — hate. The mechanical combination and separation of material particles, different in their nature, led, according to Anaxagoras' teaching, to the rise of all the diversity of natural bodies. Their definite form and harmonious structure is regulated by a principle, external to the rough matter, which Anaxagoras thought to be intellect (nous). Some commentators interpret this concept to mean very small matter; others suppose that it signifies a nonmaterial source of movement and of the formation of material bodies. Democritus rejected this dualism and returned to the monism of Phales and Heraclitus. He did not acknowledge anything as standing above matter: the world is the aggregate of atoms moving in a void. In Aristotle's opinion, matter possesses merely the capacity to acquire the character of definite bodies; form is the realization of this potentiality of matter in natural bodies, inseparable from matter, but, nevertheless, opposed to it.

One can trace over many centuries the monistic and dualistic views on living bodies, the first from Democritus and his precursors and the second from Empedocles and Anaxagoras. At times, the conflict between these viewpoints was heightened and at other times it was somewhat smoothed over.

At the end of the 19th century and at the beginning of the 20th, the differences between monistic (mechanistic) and dualistic (vitalistic) views on living beings became very sharp. The source of conflict was the success of studies of the chemistry and physical chemistry of protoplasm. These bore the deceptive assurance that the final solution of the question of vital phenomena could be expected to follow upon the application of physical analysis. In this period, particularly under the influence of J. Loch, the concept of "living substance" again attained wide popularity; the study of the elementary composition of protoplasm, the successes of synthetic organic chemistry and the possibility of reproducing some biochemical processes outside the organism for a time attracted the attention of researchers away from the fine morphology of protoplasm and the cell. It was thought (and this idea was recently supported by R. Chambers) that, by centrifuging, one could liberate protoplasm from the microscopically visible structural components and then the homogeneous "living substance" would appear — the genuine substrata as they thought, of vital phenomena. Cytologists objected to these notions since they did not consider it possible to regard the structural formations in the cell only as the product of the vital activity of protoplasm. They saw in these structures genuine organoids, i.e., active morphological formations.

Idealistic biologists at the end of the 19th century and the beginning of the 20th approached the interpretation of vital phenomena from two points of view, both unacceptable. Some, for example G. Bunge and M. Verworn, while objecting to the old vitalism of van Helmont, Boedet and Treviranus which was revived in the middle of the 19th century by Johannes Müller and J. Liebig, took a position of subjective idealism. Others, having started with mechanistic explanations of life processes and become disappointed with their results, came to the assumption that the "living machine" is directed by an all-doing, nonmaterial, and even nonspatial factor, which was called "entelechy" by H. Driesch, one of the more thoughtful representatives of this trend. Entelechy, understood as an irrational remnant of analytic fragmentation of vital processes, is indisputably the outcome of this enthusiasm for the analytical method in biology.

Neither the mechanistic nor the vitalistic view could establish scientifically the fact of a single material source of both inanimate and animate bodies, and, at the same time, explain the indisputable qualitative difference between the two. This resulted in various attempts to overcome the inadequacy of mechanistic and the mysticism of vitalistic notions. The beneficial result of these attempts, (theories of organic form (theories of gestalt), hierarchical and organismic theories, theories of wholeness, etc.), is that they more or less convincingly showed the methodological impotence of both mechanismism and vitalism. These theories themselves, however, remained the captives of idealistic conceptions of various shades.

The application of new methods of investigating cell structures (phase contrast, ultraviolet and electronic microscopy), the biochemical study of structural components separated from the cells and the cytochemical analysis of the distribution of cellular substances and their associated physiological and biochemical processes made it possible at long last to unify the concepts of form, structure and function.[140] When it was shown that form, structure and function operated in unity on the molecular and subcellular level (i.e., on the level of cell organoids), it become clear that the same unity must exist on higher levels — those of cells, tissues, organs and organisms.

The cardinal biological problem in both invariable and variable autoreproduction of organic structures possessing specific functions was until recently limited to the study of this phenomena on the highest levels accessible to macro- and microscopic investigation. Studies on the submicroscopic level were characterized by "a jump in the dark," as M. Heidenhain graphically called it, i.e., by the creation of propositions which could not be verified at that time and have not been corroborated since. To this group belongs Heidenhain's own hypothesis of protomeres, which became the property of history and one more in the long list of hypotheses, all alike founded on the assumption of the existence of elementary units of life lying beyond the limits of the resolving power of the microscope.

The idea that the autoreproducing intracellular structures of the molecular level have relatively large dimensions and under certain conditions are accessible to optical means of investigation led to more fruitful results. The objects under consideration are of interest to cytology, biochemistry and genetics. N.K. Kol'tsov first expressed a hypothesis of the structure of chromosomes based on these considerations. He proceeded from the computations of the Russian organic chemist, A.A. Kolli, connected with the first determinations of the molecular weight of proteins. Kolli supposed that protein molecules have such a significant size that the number of them in the nucleus of a spermatozoon must be very small.[150] Kol'tsov proposed that the chromosome or, more precisely, its basic (according to the hypothesis, dual) filament (chromonema or as he called it, genonema) is a bundle of long protein-nucleinic molecules. Elementary material determinents of hereditary characteristics (genes) are in Kol'tsov's view "nothing other than chemical radicals which form a large visible molecule — the chromosome."[151] In the speech, "Physico-chemical Bases of Morphology," at the opening of the Third All-Union Congress of Zoologists, Anatomists and Histologists, Kol'tsov posed a more general question: how is the synthesis — the assimilation of proteins in the cell from the solution of amino acids — realized? Even for a relatively simple polypeptide of 17 amino acids the formation of one trillion isomers is possible. When such a polypeptide is synthesized in the laboratory, the probability of receiving a given isomer is very small. "However," wrote Kol'tsov, "it is evident that in living organisms with the process of assimilation of protein from its solution of amino acids there occurs a synthesis of molecules strictly coinciding with the models already having protein molecules... The simplest hypothesis for explaining assimilation is, it seems to me, the conclusion that the process of the synthesis of protein molecules leads to crystallization around existing protein molecules or their aggregates... Hence, it follows that protein molecules share with organisms one very important characteristic."[152] Kol'tsov meant the ability of autoreproduction. To the aphorisms expressing the historical succession of discoveries of the life continuum at descending levels (Omne vivum ex vivo; omnis cellula ex cellula; omnis nucleus ex nucleo), Kol'tsov added one more: Omnis molecula ex molecula.

The original assumption on the mode of autoreproduction of molecules was that a complex organic molecule "originates from a surrounding solution only in the presence of an already prepared molecule; the corresponding radicals are placed by means of opposition at available points of the molecule which serve as a priming device and where similar radicals are found." "With the growth of a genonema bundle of molecules," wrote Kol'tsov further on, "the radicals are arranged as in crystallization, namely in those spots where (in the chromosome — L. B.) there are the same radicals... When the thickness of the genonema bundle of molecules reaches a certain limit by means of accumulation, the genonema is split lengthwise."[153]

D. Wrinch introduced an important addition to these notions in the article, "Chromosome Behavior in Terms of Protein Pattern."[154] Wrinch proposed that the long molecule, the genonema, is a chain of uniformly repeated links and that particularly in polypeptide elupeine amino acid remnants of arginine and monoamine-monocarboxylic acid are repeated. Kol'tsov considered this idea worthy of serious attention and further elaboration. The physicist, M. Del'bryuk, working with the geneticists, N. V. Timofeev-Resovskii and K. G. Tsimmer, thought that atoms or groups of atoms play an individual role in the growth of complex organic molecules, which might be called "aperiodic" crystals, in contradistinction to the usual "periodic" crystals, in which growth is accomplished by duplication of the structure.

Kol'tsov's arguments about the mechanism of assimilation by means of autoreproduction of complex organic molecules, in particular of protein molecules and of nucleinic acids, anticipates the discoveries made more than a quarter of a century later.

After a period of stagnation, which in retrospect, can be seen as a manifestation of the crisis created by the chromosome theory of heredity, rapid progress is now occurring in this area. As recently as 1959, A. Rich wrote: "In order to accomplish control over the specificity of proteins, information contained in the DNA molecule in the form of some succession of nucleotides, must pass through the molecules of RNA before it finally appears in the form of a definite succession of amino acids characteristic of some protein. If this viewpoint it correct, then it would be necessary to know how the DNA molecule makes RNA and how the RNA molecule in turn organizes amino acids. Unfortunately, at present there is still no answer to this question."[155]

At that time only a conjectural answer to this question existed — the hypothesis of A. Dounce on the duplicating mechanism for peptide chain and nucleic acid synthesis.[156] After two or three years the hypothesis received corroboration in experimental genetic and biochemical data. The introduction of laboratory-synthesized polyribonucleotides of a definite composition in a medium capable of protein synthesis outside of the organism led to the inclusion of certain amino acids in the organizing polypeptides: in some cases, of phenylalanine, in others, of tyrosine or isoleucine, etc, in short, almost all of the twenty amino acids in the composition of protein. In other words, nucleotide groupings were established by which certain amino acids are coded in a molecule of nucleic acid.[157] The investigations of M. Nirenberg, C. Ochoa and their co-workers opened the way to the deciphering of the code by which the transmission of genetic information from DNA to RNA and from the latter to proteins is accomplished.

In the light of these facts it is difficult to overestimate the historical significance of Kol'tsov's notions about the method of selective synthesis of protein molecules by which specific isomers are created in the cell from an almost infinite number of possible ones and regularly lead to a definite result.

CONCLUSION

Speculation on the causes of orderly occurrence of biological processes which had to the only conclusion possible for the organism have a history of more than twenty centuries.

Aristotle differentiated four categories of causes requiring scientific recognition: matter, form (or essence), the source of change or movement and, finally, a goal.[158] With respect to natural bodies, in particular living beings, the three last causes are, in Aristotle's conception, linked together. The moving cause of origin leads to the formation of a definite form which appears as the essence of living bodies, whose full expression is the goal or end, the completion of the process. The Greek word telos (goal) signifies both end and conclusion; Aristotle did not give an anthromorphic meaning to this concept, i.e., he did not mean a goal consciously placed (as in the intellectual activities of man). The Aristotelian classification of causes was interpreted by medieval scholastics, who gave to the concept of final, or teleologic cause,a religious-mystical meaning. Mechanistic philosophy (Descartes) denied medieval teleological (i.e., essentially theological) notions. These still remained, however, for a long time in a clearly dualistic form as the basis of the views of the old vitalism (G. Stahl) and of various modifications of French (Boedet, Bichat) and German (Blumenbach, J. Mueller) vitalists. The vitalism of a later time was based on the same dualism — the opposition of body and soul (or vital force), of acting and teleologic causes. This vitalism looked for the initiating, directing cause of structure, vital activity and development of living bodies beyond the limits of their material nature. The representatives of so-called neovitalism (Reinke, Ixkyul, Rinyano and particularly Driesch) also ascribed a purposefulness of phenomena of the material world to an extra-material agent, which they called "dominant," "system of impulses," "vital energy" or "entelechy." All these systems, essentially alike in their other-worldly "explanation" of the expediency of living nature, resisted mechanistic views, which did not attempt any explanation of this characteristic of living bodies. It was necessary to go beyond the limits of both the vitalistic and mechanistic views in search of an explanation. The operation of organic expediency was laid down in a general form by Darwin's doctrine of evolution by natural selection. Darwin showed that the source of relative expediency of living beings, lies in the survival of forms which are the most adapted to the conditions of existence, i.e., expedient in given conditions. This strictly scientific, materialistic explanation, which is to Darwin's immortal credit, was later developed in theories which derived from the study of vital phenomena on cellular and molecular levels.

K. Baer occupies a special place among those theoretical biologists of the last century who reflected seriously on the problem of the expedient structure of living beings and the directed character of individual development. He strongly objected to the views of the vitalists of his time and insisted upon the need to consider the fact that all the conditions and changes of a healthy organism provide for its future needs. Although he did not accept the Darwinian explanation of the occurrence of the expedient organization of living beings, Baer, limiting himself for his part to general arguments, did not propose any other theory. The concept of necessity (corresponding to Aristotle's moving cause) and the concept of teleology (also corresponding to the Aristotelian concept of end or conclusion) should not, in Baer's thought, be opposed to each other since the goal or conclusion of a process is attained because of necessity.[159]

In Baer's thought, as in Aristotle's, the concept of the unity of means and goal in the organization and development of living begins, i.e., of the acting and of the teleologic principles, did not take the form of a monistic viewpoint. One hundred years ago, when Baer published his articles about expediency in animate nature, the necessary factual data for its scientific substantiation did not exist. It was only in recent times that it was possible to corroborate that the mechanism which provides for the continuity of life includes the source of reproduction, in changed or unchanged form, of the specific material basis of vital phenomena.

The achievements of contemporary science removes the necessity of searching for something supraordinate to organic structures as a source of their biochemical specificity. In other words, bases for a dualistic interpretation of the nature of living beings are disappearing. Twenty-two centuries after their proclamation, the Aristotelian concepts of "matter" and "form," of "acting" and "teleologic" causes are ready to join in indissoluble unity.

Notes

[1] In Plutarch's words, Thales "learned from the Egyptians to consider water as the original substance and the basis of all things."— Istoriya filosofii, Vol. 1, p. 42. [Russian translation. 1957.]

[2] Lucretius. On the Nature of Things (1, 835-838).

[3] Ibid. (1, 843-844).

[4] Engels, F. Dialectics of Nature. [Russian translation]. — In: K, Marx and F. Engels. Works, Vol. 20:367-368.

[5] Cited from Makovel'skii, A.O. Drevnegrecheskie atomisty (Ancient Greek Atomists), p. 210. Baku. 1946.

[6] Plato

[7] Aristotle. Metaphysics, (1028 b 3-35).

[8] Ibid., (1028b 35-1029b6).

[9] Aristotle. De partibus animalium, (640b).

[10] Ibid.

[11] Karpov, V.P. Naturfilosofia Aristotelya (Aristotle's Natural Philosophy). — In: Voprosy filosofii i psikhologii, Book 109:544. 1911.

[12] Aristotle. De partibus animalium, (646a).

[13] Ibid., (646b).

[14] Ibid., (642 a).

[15] Karpov, V.P., p. 544.

[16] Aristotle. De partibus animalium, (640a).

[17] Ibid., (640b).

[18] Aristotle. Metaphysics (1041b 5-33).

[19] Lucretius. On the Nature of Things (I, 814-822).

[20] Ibid., (II, 479-480).

[21] Ibid., (II, 695-697).

[22] Ibid., (II, 700-701).

[23] Meslier, J. Zaveshchanie (Testament). [Russian translation edited by A. Deborin. 1925.]

[24] Lamettrie, J. Izbrannye sochineniya (Selected Works). [Russian translation. 1925.]

[25] Diderot, D. Filosofskie printsipy materii i dvizheniya. Izbrannye sochineniya (Philosophical Principles of Matter and Movement. Selected Works). [Russian Translation. 1926.]

[26] Ibid., p. 133.

[27] Lomonosov, M.V. Vvedenie v istinnuyu fizicheskuyu khimiyu (1752-1754). Polnoe sobranie sochinenii (Introduction to True Physical Chemistry (1752-1754). Complete Works), Vol. 2:535-555. Moskva-Leningrad. 1951.

[28] Buffon, G. Histoire naturelle, Vol. 2:40. Paris. 1749.

[29] Maupertuis, P. Venus physique. Seconde partie. Variété dans l'espèce humaine. 7th Edition, pp. 134-135. Genève. 1780.

[30] Darwin, E. Zoonomia or the Law of Organic Life, Vol. 2. Ch. 39. London. 1794.

[31] Fontana, F. Traité sur le venin de la vipère, sur les poisons américains, sur le laurier-cerise et sur quelques autres poisons végétaux. On y a joint des observations sur la structure primitive du corps animal etc., Vol. 2:254-255. Florence. 1781.

[32] Purkyně, J.E. Symbolae ad avium historiam ante incubatonem.— Bratislavae: 1825. (Opera selecta, pp. 1-25), Prague. 1948).

[33] Mirbel, C.F. Recherches sur le marchantia. Paris. 1831-1832.

[34] Brown, R. On the Organs and Mode of Fecundation in Orchidae and Asclepiadeae.— Trans. Linn. Soc., Vol. 6:685-745. 1883.

[35] Purkyně, J.E. Über den Bau der Magen-Drüsen und über die Natur des Verdauungsprozesses. Bericht über die Vers. deutsch. Naturf. und Ärzte in Prag. 1837. (Opera selecta. pp. 109-111).

[36] Meyen, F.J.F. Anatomisch-physiologische Untersuchungen über den Inhalt der Pflanzenzellen. Berlin. 1828.

[37] Schwann, Th. Mikroskopische Untersuchungen über die Übereinstimmung in der Struktur und Wachstum der Tiere und Pflanzen. Berlin. 1939.

[38] Schleiden, M.J. Beiträge zur Phytogenesis.— Arch. Anat. Physiol. 1838.

[39] Dujardin, F. Recherches sur les organismes inferieures. Ann. sci. nat. Zool. Séries 2, Vol. 4:367-368. 1835.

[40] Purkyně, J. (Purkinje) Über die Analogien in den Strukturelementen des tierischen und pflanzlichen Organismus. — Übersicht der Arbeiten und Veränderungen der Schlesischen Gesselschaft, f. vaterländ. Kultur im Jahre 1839, S. 81-82. Breslau. 1840. (Opera selecta, pp. 114-115).

[41] Mohl, H. Über die Saftbewegung im Inneren der Zelle.— Bot. Ztg., Jg. 4., S. 73-79, 89-94. 1846.

[42] Raspail, F. Dévelopment de la fécule dans les organes de la fructification des céréales, et analyse microscopique de la fécule, suivie d'expériences propres à en expliquer la conversion en gomme.— Ann. sci. nat., Vol. 6:224-239, 383-427. 1825.

[43] Pierce, E.H. Gistokhimiya teoreticheskaya i prikladnaya (Theoretical and Applied Histochemistry). Russian translation by A.N. Belozerskii. 1962.

[44] Chevreul, M. Considérations générales sur l'analyse organique et sur ses applications, p. 238. Paris. 1824.

[45] Ibid., p. 241.

[46] Kölliker, A. Gistologiya, ili uchenie o tkanyakh cheloveka (Histology or Teaching about Human Tissues). Russian translation from 4th Edition by V. Kovalenskii. 1865.

[47] Virchow, R. Patologiya, osnovannaya na teorii yacheek (tsellyulyarnaya patologiya), v primenenii k mikroskopicheskoi anatomii normal'nykh i nenormal'nykh tkanei (Pathology, Based on Cell Theory (Cell Pathology), with Respect to the Microscopic Anatomy of Normal and Abornal Tissues), Russian translation from German. 1859.

[48] Schultze, M. Über Muskelkörperchen und das, was man eine Zelle zu nennen habe. — Arch. Anat. Physiol., S. 3-4. 1861.

[49] Ibid., S. 11.

[50] Brücke, E. Die Elementarorganismen. — Sitzungsber. Akad. Wiss. Wien. Math-nat. Cl., Bd. 44, Abt. 2, S. 2381 (footnote). 1861.

[51] Ibid., S. 385.

[52] Ibid., S. 386.

[53] Huxley, T.H. On the Physical Basis of Life. — In: University Series, No. 1, pp. 7-35. Charles C. Hatfield, New Haven. 1870.

[54] Ibid., p. 13.

[55] Ibid., p. 17.

[56] Ibid., p. 18.

[57] Ibid., pp. 19-20.

[58] Bernard, Cl. Leçons sur les phénomènes de la vie communs aux animaux et aux vegetaux, Vol. 1:33. Paris. 1878.

[59] Ibid., p. 41.

[60] Engels, F. Anti-Dühring [Russian translation]. — In: K. Marx and F. Engels, Works. Vol. 20:610-611.

[61] Hegel, G. — Entsiklopediya filosofskikh nauk, Part 1. Works, Vol. 1:136. 1929.

[62] Engels, F. Dialectics of Nature. [Russian translation]. — In: K. Marx and F. Engels Works. Vol. 20:610-611.

[63] At the end of his concluding lecture, Cl. Bernard remarks: "Between two schools, one of which considers vital phenomena to be completely different than physicochemical phenomena, and the other, which regards them as completely identical, there is room for a third trend, physical vitalism, which takes into account that vital manifestations have somethingspecific and that general forces act in agreement: the latter element rules physical nature, but topography has a vital character." Bernard, Cl. Leçons sur les phénomènes de la vie, Vol. 2:524. Paris. 1879.

[64] Huxley, T.H. On the Physical Basis of Life. — In: University Series, No. 1:7-35. Charles C. Hatfield, New Haven. 1870.

[65] Delage, Y. La structure du protoplasma, et les theories de l'hérédité et les grands problemes de la biologie. p. 878. Paris. 1895.

[66] Hofmeister, W. Die Lehre von der Pflanzenzelle. — Handbuch physiol. Bot., Bd. 1, S. 1. 404 S. Leipzig. 1867.

[67] Ibid., S. 59-60.

[68] Remak, R. Neurologische Erläuterungen. — Arch. Anat. Physiologie, S. 463-472. 1864.

[69] Eberth, C.J. Zur Kenntniss des feineren Bau der Flimmerepithelien. — Arch. pathol. Anat., Bd. 35:477-478. 1866.

[70] Stuart, A. Über die Flimmerbewegung. — Diss. S. 45. Dorpat. 1857.

71 Fromann, C. Zur Lehre von der Struktur der Zelle.—Jena. Zeitschrift, Bd. 9:280-298. 1875.

72 Velten, W. Bewegung und Bau des Protoplasmes.— Flora, Jg. 56: 82-88, 113-128. 1873.

73 Fayod, V. Über die wahre Struktur des lebendigen Protoplasmas und Zellmembran.— Naturwissenschaftliche Rundschau, Jg. 5:81-84. 1890.

74 Baranezky, J. Die Kernteilung in den Pollenmutterzellen einiger Tradeskantien.— Botanische Zeitung, N. 38:241-248, 265-274, 281-296. 1880.

75 Heitzmann, J. Untersuchungen über das Protoplasma. I. Bau des Protoplasmas.— Proc. Akademie der Wissenschaften Wien, Math. -phys. Cl., Bd.67, Abt. 3:100-115. 1873.

76 Schaeffer, E. On the Structure of Ameboid Protoplasm, etc.— Proc. Royal Society. London, Vol. 49:193-198. 1891.

77 Carnoy, J. La Biologie cellulaire, Ètude comparèe de la cellule dans les deux règnes. 271, Lierre. 1884.

78 Schleicher, W. Die Knorpelzellteilung.— Arch. mikroscop. Anatomie, Vol. 16:248-300. 1878.

79 Flemming, W. Zellsubstanz. Kern- und Zellteilung. VIII+424, S. 1882.

80 Bütschli, O. Studien über die ersten Entwicklungsvorgänge der Eizelle, die Zellteilung und die Conjugation der Infusorien.— Abh. Senkenberg. Naturf. Gesellschaft, N. 10. 1875. Quoted from O. Bütschli. Untersuchungen über mikroskopische Schäume und das Protoplasma. Versuche und Beobachtungen zur Lösung der Frage nach den physikalischen Bedingungen der Lebenserscheinungen. 234 S. Leipzig. 1892.

81 Bütschli, O. Untersuchungen über mikroskopische Schäume und das Protoplasma. Versuche und Beobachtungen zur Lösung der Frage nach den physikalischen Bedingungen der Lebenserscheinunge. 234 S. Leipzig. 1892.

82 Rhumbler thought it better to compare protoplasm with an emulsion, rather than with foam, cells of which are filled with air. He proposed to call such a structure an "emulsoid, frothy mixture" or the etymologically unfortunate Latin-Greek term, "spumoid." (Rhumbler, L. Das Protoplasma als physikalisches System.—Ergebnisse der Physiologie, Bd. 14:474-617. 1914.

83 Gurwitsch, A.G. O yavleniyakh regulyatsii v protoplazme (On the Phenomena of Regulation in Protoplasm).— Trudy S. Peterburgskogo Obshchestva Estestvoispytatelei, Vol. 37:139-190. 1908.
In the experiments, the eggs of echinodermata and amphibia were intensively centrifuged for a short time; as a result of this, the structure of the ooplasm was sharply dislocated by a stratification which corresponds to the specific gravity of separate components and by coarse vacuolization resulting from the rapid centrifugal displacement of granules of yolk. In a number of cases the restitution of the cytoplasm soon appeared, so that mitotic division could take place practically normally. Later, in summing up the results of these experiments, A. G. Gurwitsch formulated an important generalization, which could be called the kinetic, or as Gurwitsch preferred to call it, the "physiological theory of protoplasm." At the basis of this theory is the notion of unstable constellations of protoplasm, of molecules, which are temporarily held in definite mutual positions; the unstable condition

of such constellations is supported by a flow of energy of exothermic chemical reactions of the metabolism. Gurwitsch called the processes of continuous renewal of the molecular composition of unstable constellations of protoplasm avestigial processes, i.e., processes which do not leave a trace. In the case of combined molecular reaction, a stable molecular constellation arises — a vestige of a pre-existing unstable constellation, appearing as a rudiment of one or another stable protoplasmic structure (The physiological theory of protoplasm is more fully established in the book: Gurwitsch, A. and L. Vvedenie v uchenie o mitogeneze (Introduction to the Theory of Mitogenesis). Ch. 9:77-100. Moscow. 1958.

[84] Künstler, J. Recherches sur la morphologie des flagellés. — Bull. sci. France et Belg., Vol. 20:399-515. 1889.

[85] Bütschli, O. S. 121.

[86] Ibid.

[87] Künstler, J. Contribution à l'étude des flagellés. — Bull. soc. Zool. France, Vol. 7:112. 1882.

[88] Martin, H. Recherches sur la structure de la fibre musculaire striée et sur les analogies de structure et de fonctions entre le tissue musculaire et les cellules à batonnets. — Arch. d'anatomie normale et pathologique, pp. 465-510. 1882.

[89] Pfitzner, W. Beiträge zur Lehre vom Bau des Zellkerns und seinen Teilungserscheinungen. — Arch. für mikr. Anatomie, Bd. 22:618-688. 1882.

[90] Brass, A. Biologische Studien. 1. Die Organisation der tierischen Zelle. H. 1. 2. Halle. 1883-1884, cited from Delage, Y. La structure du protoplasm, et les théories de l'hérédité et les grands problèmes de la biologie.

[91] Vejdowský, F. Entwicklungsgeschichtlike Untersuchungen, H. 1. Reifung, Befruchtung und die ersten Teilungsvorgänge des Rhynehelmis-Eies. 166 S. Prague. 1886.

[92] Altmann, R. Die Elementarorganismen und ihre Beziehungen zu den Zellen, 145 S. Leipzig. 1890.

[93] Béchamp, A. Les microzymas dans leur rapport avec la fermentation et la physiologie et pathologie. Examen de la panspermie atmosphérique continue ou discontinue, morbifère et non morbifère. XXXVIII+992 p. Paris. 1883.

[94] Delage, Y. La structure du protoplasm, et les theories de l'hérédité et les grand problemès de la biologie. p. 422. Paris. 1895.

[95] Spencer, H. The Principles of Biology, p. 129. Translated into Russian by A. Gerd. 1870.

[96] Haake, W. Gestaltung und Vererbung. Eine Entwicklungsmechanik der Organismen. VI+337 S. Leipzig. 1893.

[97] Haeckel, E. Über die Wellenzeugung der Lebensteilchen oder die Perigenesis der Plastidule. Gemeinversständl. Vortrag und Abhandlung aus dem Gebiete der Entwicklungslehre, Bd. 2:31-97. Bonn. 1902.

[98] Delage, Y. La structure du protoplasma, et les théories de l'hérédité et les grands problèmes de la biologie. p. 464. Paris. 1895.

[99] Erlsberg, L. Regeneration, or the Preservation of Organic Molecules. A Contribution to the Doctrine of Evolution. — Ass. Adv. Sci. Hartford Meeting, pp. 87-103. 1874. Erlsberg, L. On the Plastidule-Hypothesis. — Ibid., Buffalo Meeting, pp. 178-187. 1876.

[100] Hanstein, J. Das Protoplasma als Träger der pflanzlichen und tierischen Lebenserscheinungen. 171 S. Heidleberg. 1880. Hanstein's evolutionary views also have an idealistic character. He was an anti-Darwinist, an advocate of polyphyletic views. "Morphological similarity," in his words, "is the necessary result, on the one hand, of a similarity in primordia (Urkeime) of their characteristic developmental plans, and, on the other hand, a result of the basic morphological regularity, which occurs as similar needs give rise to similar structures" (J. Hanstein, op. cit. pp. 183-184). Hanstein later speaks of the primary creative act (Urschöpfungsakt) as the source of the individuality of living beings and the initial moment of the development of the psyche.

[101] Berthold, G. Studien über Protoplasma-Mechanik, S. 81. 332 S. Leipzig. 1886.

[102] Altmann, R. Die Elementarorganismen und ihre Beziehungen zu den Zellen. 145 S. Leipzig, 1890.

[103] Weisner, J. Die Elementerstruktur und das Wachstum der lebenden Substanz. 279 S. Vienna. 1892.

[104] Heidenhain, M. Allgemeine Anatomie der lebendigen Masse. Abt. 1, Plasma und Zelle, S. 485. 5065. Jena. 1907.

[105] Aristotle. De Generations Animalium (721b).

[106] Ibid., (722a).

[107] Ibid., (734a).

[108] Hösch. Versuch einer neuen Zeugungslehre. 1801. Cited from Delage, Y. La structure du protoplasma, et les théories de l'hérédité et les grands problèmes de la biologie, p. 550. Paris. 1895.

[109] "I... recognize with pleasure from Darwin's letters," wrote K.A. Timiryazev, "that he himself pronounced such a strict judgment on it (the theory of pangenesis): 'It is all rubbish to speculate as I have done.'" (Timiryazev, K.A. God itogov i pominok. Sochineniya. (A Year of Achievements andFeasts. Works). Vol. 9:102. Moskva. 1939. Citation from a letter of Darwin to Huxley. In: The Life and Letters of Ch. Darwin, Vol. 3:45. London. 1888.

[110] Brooks, W.K. The Law of Heredity. A Study of the Cause of Variation and the Origin of Living Organisms. Baltimore. 1883.

[111] From this entirely unfounded assertion, Brooks made far reaching, fantastic deductions. He thought that in human society the intellectual and moral differences of the sexes reflect the differences between "embryos" and "gemmules." In his opinion, woman is the conservative historical element of society; she preserves established ideas and old mores. Man, on the other hand, is impulsive and less stable and is therefore capable of creating new cultural values.

[112] Hallez, P. Pourquoi nous ressemblons à nos parents? Paris. 1886.

[113] Gaule. Über die Bedeutung der Cytozoen für die Bedeutung der tierischen Zellen.— Biol. Ztbl., Bd. 6:345-351. 1886.

[114] Naegeli, C. Mechanisch-physiologische Theorie der Abstammungslehre. Munich-Leipzig. 1884.

[115] Kölliker, A. Die Bedeutung der Zellkerne für die Vorgänge der Vererbung.— Zeitschrift für wissenschaftliche Zoologie, Bd. 42:1-46. 1885.

[116] Hertwig, O. Der Kampf um Kernfragen der Entwicklungs und Vererbungslehre, S. 26-27. Jena. 1903.

[117] Hertwig, O. Beiträge zur Kenntnis der Bildung, Befruchtung und Teilung des tierischen Eies. — Morphologisches Jahrbuch, Bd. 1. 1875.

[118] Hertwig, O. Das Problem der Befruchtung und der Isotropie des Eies. Eine Theorie der Vererbung. — In: Sitzungsber. Jena. Ges. Med. Naturwiss. 28 November, 1884; Jena Zeitschrift., Bd. 19, Suppl. H. 1, S. 76-78. 1885.

[119] Strasburger, E. Neue Untersuchungen über den Befruchtungsvorgang bei den Phanerogamen als Grundlage für eine Theorie der Zeugung. XI+176 S. Jena. 1884.

[120] Jäger, G. Zur Pangenesis. Kosmos, Bd. 2 (Gratulationsheft zum 70-ten Geburtstage Ch. Darwin's), S. 377-385. 1879.

[121] Rauber, A. Formbildung und Formstörung in der Entwicklung von Wirbeltiere. — Morphologisches Jahrbuch, Bd. 6. 1880.
Nussbaum, M. Die Differenzierung des Geschlechts im Tierreich. — Arch. mikr. Anat., Bd. 18. 1880. The works of Jäger, Rauber and Nussbaum are cited in Weismann, A. Das Keimplasma. Eine Theorie der Vererbung. XVII+628 S. Jena. 1892.

[122] de Vries, H. Intracellular Pangenesis. VI+212 p. Jena. 1889.

[123] Hertwig, O. Allgemeine Biologie. Jena. 1909. Translated into Russian by A.S. Dogel. 1911.

[124] Verworn, M. Prinzipienfragen in der Naturwissenschaft. Jena. 1905. Cited from Hertwig, O. Der Kampf um Kernfragen der Entwicklungs und Vererbungslehre, S. 44-45. Jena. 1903.

[125] Conclin, E. The Mechanism of Heredity. — Science, Vol. 27:89-99. 1908.

[126] Yudin, A. L. Problema geneticheskogo koda: nekotorye noveishie dostizheniya (The Problem of the Genetic Code: Some Very Recent Achievements). — Tsitologiya, Vol. 4, No. 2:101-108. 1962;
Olenov, Yu. M. O peredache informatsii ot DNK k belkam (On the Transfer of Information from DNA to Proteins). — Ibid., pp. 109-116.

[127] Delage, Y. La structure du protoplasma, et les theories de l'hérédité et les grand problèmes de la biologie, p. 751. Paris 1895.

[128] de Vries, H. Plasmatische Studien über die Vacuolen. — Jahrbuch für wissenschaftliche Botanik Bd. 16, S. 465-592. 1885.

[129] Boveri, Th. Die Befruchtung und Teilung des Eies von Ascaris. Zs., Bd. 22, S. 685-882. Jena. 1888.

[130] Delage, Y. La structure du protoplasma, et les theories de l'hérédité et les grand problèmes de la biologie. p. 752. Paris 1895. The insight of these judgments can only now be fully appreciated when electron microscopic investigations have showed the presence of numerous intracellular membranes of different construction (cf. for example, F. Shestrand. The Fine Structure of Protoplasm. Organization of the System of Membrane Layers. — In: Sbornik "Sovremennye problemy biofiziki," Vol. I. Translated into Russian by Yu. I. Lyashkevich, pp. 46-57. Moskva. 1961.

[131] Hofmeister, F. Die Chemische Organisation der Zelle, S. 9, Braunschweig. 1901.

[132] Ibid., S. 11.

[133] Ibid., S. 18.

[134] Ibid., S. 22-23.

[135] Ibid., S. 24.

[136] Ibid., S. 25.

[137] Ibid., S. 26.

[138] Ibid., S. 29.

[139] Höber, R. Physikalische Chemie der Zelle und der Gewebe, Aufl. 5, S. 817-822. Leipzig. 1924.

[140] Gurwitsch, A.G. O yavleniyakh regulyatsii v protoplazme (On the Phenomena of Regulation in Protoplasm). — Trudy S. Peterburgskogo Obshchestva Estestvoispytatelei, Vol. 37:139:190. 1908.

[141] Wilson, E.B. The Cell in Development and Heredity. 3rd Edition revised and enlarged. XXXVII+1232 p. — Macmillan, New York. 1940.

[142] Warburg, O. Über die Wirkung der Struktur auf chemische Vorgänge in Zellen. 21 p. Jena. 1913.

[143] Loeb, J. Dinamika zhivogo veshchestva (The Dynamics of Animate Matter). — Translated by V. V. Zavyalova. 1910.

[144] "Corpora non agunt nisi soluta." (Höber, R. Physikalische Chemie der Zelle und der Gewebe. S 10. Leipzig. 1902).

[145] Dutrochet, H. Mémoires pour servir à l'histoire anatomique et physiologique des végétaux et des animaux. Vol. (De l'endosmose), pp. 1-99. Paris. 1837.

[146] Traube, M. Experimente zur Theorie der Zellenbildung und Endosmose. — Arch. Anat. Physiol., S. 87-165. 1867.

[147] Pfeffer, W. Osmotische Untersuchungen. Studien zur Zellmechanik. VIII+236 S. Leipzig. 1877.

[148] de Vries, H. Eine Methode zur Analyse der Turgorkraft. — Jahrbücher für wissenschaftliche Botanik, Bd. 14:427-601. 1884.

[149] The history of the interrelationship of these concepts is dealt with in another report of the present series: Blyakher, L.Ya. Sootnoshenie formy i funktsii (The Correlation of Form and Function). — (Etyudy po istorii morfologii. 4) (Studies in the History of Morphology). — Trudy Instituta Istorii Estestvoznaniya i Tekhniki, Vol. 40:118-156. 1962. and in Blyakher, L.Ya. Ocherk istorii morfologii zhivotnykh (Survey of the History of Animal Morphology). 258 p. — Izdatel'stvo AN SSSR. 1962.

[150] Kolli, A.A. Mikroorganizmy khemicheskoi tochki zreniya (Microorganisms from the Chemical Viewpoint). — In: Dnevnik 9 S"ezda Russkikh estestvoispytatelei i vrachei (Appendix). 1894.

[151] Kol'tsov, N.K first published this hypothesis in the article, "Biologiya," (Biology) in the first edition of Bol'shaya Sovetskaya Entsiklopediya, Vol. 6, p. 329:296-337. 1927.

[152] Kol'tsov, N.K. Organizatsiya kletki (Organization of the Cell). — In: Sbornik eksperimental'nykh issledovanii, statei i rechei 1903-1935, pp. 461-490, quotation on pp. 482-483. Moskva-Leningrad. 1936.

[153] Kol'tsov, N.K. Nasledtsvennye molekuly (Hereditary Molecules). Speech at annual meeting of Moskovskoe obshchestvo ispytatelei prirody. January, 1935. — In: Kol'tsov, N.K. Organizatsiya kletki (Organization of the Cell), pp. 585-622, quotation on pp. 618-619.

[154] Wrinch, D.M. Chromosome Behavior in Terms of Protein Pattern. — Nature, Vol. 134(3399):978-979. 1934.

[155] Rich, A. Molekulyarnaya struktura nukleinovykh kislot (Molecular Structure of Nucleinic Acid). — In: Sbornik "Sovremennye problemy biofiziki," Vol. 1:246-256, quotation on p. 249. Moskva. 1961

[156] Dounce, A.L. Duplicating Mechanism for Peptide Chain and Nucleic Acid Synthesis. — Enzymologiya, Vol. 15:251-258. 1952.

[157] Nirenberg, M.W. and J.H. Matthaei. The Dependence of Cell-free Protein Synthesis in E. Coli upon Naturally Occurring or Synthetic

Polyribonucleotides. — In: Proc. National Academy of Sciences, Vol.47: 1588-1602. 1961; Ochoa, S. et al. Synthetic Polynucleotides and Amino Acid Code. Ibid., Vol. 47:1936-1942. 1961; Vol. 48:63-68. 1962.

[158] Aristotle. Physics, (194). — Second Analytics, (94a-95a). Aristotle's teaching about causes is stated in Karpov, V. P. Naturfilosofiya Aristotelya (Aristotle's Natural Philosophy). — In: Voprosy filosofii i psikhologii, pp. 570-574. 1911.

[159] Baer states these views in Baer, K. E. v. Über den Zweck in den Vorgängen der Natur. Abt. 1. Über Zweckmässigkeit oder Zielstrebigkeit überhaupt. Reden und kleinere Aufsätze..., Bd. 2:49-105. Saint Petersburg. 1854; Über Zielstrebigkeit in den organischen Körpern insbesondere. Ibid., pp. 170-234. For a discussion of the arguments in these articles, see Blyakher, L. Ya. Istoriya embriologii v Rossii (XVIII-XIX centuries) (History of Embryology in Russia((18th-19th centuries)), pp. 281-294. Moskva. 1955.

L.V. Belousov

ORIGIN, DEVELOPMENT AND PROSPECTS OF THE THEORY OF THE BIOLOGICAL FIELD

The following considerations justify an attempt to describe in general lines the development of Gurwitsch's theory of the biological field.

It has become almost commonplace to speak about the weakness of biological theories which lag far behind the massive accumulation of experimental data. Clearly, various branches of biology have different requirements. We shall be concerned mainly with two branches: the so-called molecular biology, which has developed as a result of the joint effort of biophysicists, physicists and biologists, and the branch called causal embryology. These two branches make many common demands on theory.

Molecular biology studies the behavior of molecules in living systems, and the mere fact of its existence indicates that the behavior of molecules in the living cell is qualitatively different from that in vitro. In very general lines, the difference consists of certain limitations of the degrees of freedom. We shall not dwell upon the factors causing these limitations; we shall mention here only the imminent need in molecular biology for concepts of direction and order of molecular motion (or deformations), regardless of whether they are determined by a stable membrane or "cytoskeletal" structures, or by factors of a more dynamic nature.

Embryology deals with factors and systems acting at the cellular level of organisms, and is thus constantly preoccupied with the characteristics of the processes, direction and order. These features are found in all cases of organized development dealing either with space-determined cellular movement or with local cell multiplication, or with the regular orientation of mitotic and cellular axes. An additional, specifically embryological feature is the need for the concept of "the whole." This need arises from the way the embryonal activity is regulated, and shows that at least certain properties of the cell are determined, not only by their internal characteristics, but by their location within the whole embryo or primordium.

Authors of many embryological theories have attempted to elucidate in a general manner the factors of order and direction in cellular processes and the influence of "the whole" on the elements. In an incomplete list of such theoretical concepts are the "tropisms" of Leb, the "gradients" of Child, the "morphogenic fields" of Weiss, the "field-gradiant" of many English authors and the "organicism" and "cholism" of Bertalanffy and Woodger. We shall not analyze these concepts here, but without belittling their value, we must claim that some of them (especially the "gradients" of Child) are no more than compilations of empirical data, and are not valid, comprehensive theories. Other, more abstract concepts deviated from the mainstream of experimental investigations and do not affect it; often they were not even applied in practical work by the authors themselves.

Gurwitsch's theory of the biological fields is usually included in this last category and is regarded by the few scientists familiar with it as a theory of natural philosophy in the least flattering sense of the term — a vague, almost mystical theory, which explains nothing and appears, at the present level of the development of biology, as useless, outmoded and even refutable.

With this background we set ourselves the following tasks:

1. To attempt a brief analysis of the entire 50-year-old history of the theory of the biological field, paying special attention to its rational and empirical origins, and to those central problems of biology upon which it was meant to shed light.
2. To try to show that well-known advances in biology have not refuted or nullified the theory of the biological field, but, on the contrary, have identified certain obvious gaps in our knowledge. The theory of the biological field was designed to fill these gaps, and, indeed, it would seem that they could not have been filled otherwise. Modern science tends to overlook these gaps and to be satisfied with advances in fields which may not be more important, but are more readily developed.

In short, we shall try to show that useful research work can proceed from "the field" theory, although any being performed today is largely oblivious of it. We shall also show that this theory is applicable to all branches of biology which study order and direction in the behavior of elements and, primarily, to embryology and molecular biology.

Gurwitsch's theory of the biological field was first developed to answer questions in embryology and therefore, employed embryologic experimental material /1, 2, 3, 4/. Only later /5, 6/ was it modified to include many other biological phenomena which can be dealt with at the molecular level. The first, very general and abstract formulation of the field principle was preceded by a thorough, logical analysis of the concepts and theories prevailing in embryology in the first decade of the twentieth century.

In the first, great revolutionary advances in the mechanics of development, Gurwitsch found three interrelated problems which still lacked satisfactory solution:

1. The relation between "the whole" and "the part" in the process of development. (As is well known, Driesch, who first formulated this problem, refrained from giving it a scientific solution.)
2. The realization of hereditary potentialities in the course of embryonal development or the realization of hereditary (species) characteristics in ontogenesis.
3. The creation of general patterns, characterizing sufficiently long periods of development, i. e., the problem of resynthesis. Experimental embryology could not solve this problem as it began with a fragmentation of the process of development.

Gurwitsch proposed entirely original methods for the solution of these problems. The concepts elaborated by him led to a formulation of the main principles of "the field" theory.

1. ORIGINS OF THE FIELD THEORY

Study of the relation between "whole and "part"

Since the end of the last century it has been pointed out in botanical works that, in many organisms and at many stages of development, the location of the cell or of the cellular complex within the whole largely determines its fate. Following the experiments of Driesch, this idea acquired a general biological importance and, ever since, it has been repeatedly confirmed by experiments on transplants, shifts, sections and concrescences of rudiments. Neither the presence of organisms nor the occurrence of developmental stages, which seem to contradict this conclusion in experiments where regulated activity is not observed, or where the investigation divides a certain phenomenon of regulation into its component parts, invalidates the universality of the principle.

In the article "Heredity as a process of realization" /1/, Gurwitsch emphasizes the main point of Driesch's conclusions: "certain parameters which characterize processes taking place in elements can be explained only by eliminating those factors which refer exclusively to the whole (possibly, the ones in process of formation). They (the parameters) include certain constants which have meaning only in relation to 'the whole' and which, if one can put it so, cannot be expressed in the language of the elements" /1, p. 462/.

This statement stresses an important aspect of the process of regulation which is usually overlooked. If some of the factors determining the fate of element A are not found inside this element, then the conclusion which is generally drawn is that these factors are contributed by another (usually a neighboring) element, B. This is, however, only half-true, because, whatever the actual relationships between elements in the regulatory process, the resultant fate of the element is a function of coordinates characterizing it as a whole, i.e., a function of magnitudes which can be expressed only in relation to the entire complex of cells participating in the process. This becomes completely clear if we refer to any regulation which is operative during disruption of a mesenchymal or epithelial embryonal tissue.

Driesch considered the formulation of these "harmonically equipotential systems" as the highest ideal in the analysis of regulatory processes. His efforts to demonstrate the validity of this "ideal" led him to skepticism and placed his investigations outside experimental embryology and rational science, in general. For Gurwitsch, on the contrary, the conclusions of Driesch were only a first step toward the analysis of regulatory processes. We did not adopt the classical methods of studying development, because in his opinion, "the experiment in the field of embryology has a somewhat peculiar character: the results of an almost 60-year period of experimental work are impressive in the sense that they destroy preconceived views, but are weak in the creation of new concepts" /7, p. 93/. For Gurwitsch, "the study of morphogenesis is equivalent to an investigation of the relationships between part and whole" /2, p. 460/. The most difficult and the most important thing is to give the concept of "the whole" such a meaning that: 1) it becomes a "working concept" i.e., usable in practical research work, and 2) it can be applied essentially to any biological subject, and not only to the ideal regulatory systems of Driesch.

"The purely embryological arguments of Driesch . . . are based upon certain facts which are, to an extent, a happy coincidence in research. Would his arguments in favor of the 'factors of the whole' and the study of their properties be seen as vulnerable had he not worked with such ideally regulated material as the eggs of sea-urchin, of Tubularia and of Clavellina? . . .

"I was stimulated by the brilliant work of Driesch to attempt to open a new line of investigation which would provide additional support for the special cases demonstrated by Driesch and, further, provide a view of the 'factor of the whole' as a reality, the properties and processes of which should be studied like the properties and processes of any object. In this attempt, I do not wish to refer to special forms and little-known processes but, on the contrary, I would like to analyze the participation and means of action of the factors of the whole on the most inconsequential and best-known forms" /3, p. 386/.

Because of Driesch's influence, the concept of "the whole" still retains for many investigators an extremely dim, and sometimes mystic, aspect. Gurwitsch has done a great amount of analytic work on the concept of "the whole." Ignorance of his work is perhaps one of the main causes for the prejudiced attitude toward "the field" theory

The first stage of this work began with the application of statistical methods to morphogenetic problems. The following were the premises: "In an entirely objective, unprejudiced examination of an embryo, or of a sufficiently developed primordium, consisting of more or less homogeneous cells, one feels justified in regarding this primordium as a 'collective form.' Two established characteristics warrant this approach: 1) the behavior of the component cells which form the embryo, in relation to the whole (the collective form), and 2) the high degree of autonomy of each of the components. If we add a third consideration — the homogeneity (near equipotentiality) of the primordial elements in the early stages — these facts provide a basis for both the main formulation of the problem and the general methodics of analytical embryology" /8, p. 96/.

A simple and general problem which is, however, completely foreign to classical embryology, thus becomes not only understandable, but even soluble. The problem is the possible degree of interrelation (or of independence) between the elementary morphogenetic processes, on the one hand, and "the whole," on the other, in different examples of organized morphogenesis. Or stated another way: "what is the minimal degree of 'accuracy' of elementary processes compatible with the 'accuracy' of 'the whole' formed by them?" In specific cases, the concepts of "connection" and "accuracy" can receive an exact quantitative expression.

In his work, "On determination, rate-setting and chance in ontogenesis" /9/, Gurwitsch analyzes several cases of morphogenesis of "the whole" which are geometrically accurate (ideally symmetric): the gastrula of sea-urchin, the young root of onion, the crystalline lens and cornea of the eye and the chick embryo. It is noteworthy that the growth of these materials is only connected with cell multiplication. In order to maintain the precise symmetry of an object during growth, the number of mitoses in the right and left halves must be the same, and the probability of mitosis in a given half must be 0.5 of the probability of onset of mitosis wherever it might be. What deviations from this ideal probability are actually found in simultaneously occurring mitoses?

The deviations from the 1:1 mitotic ratio in mitoses taking place simultaneously in the left and right halves were measured on fairly large sampling, as well as the frequency of each deviation. Graphic representation of these data, i.e., plotting of the magnitude of deviation against its frequency resulted in a curve analogous to the Gaussian curve of normal error distribution. Comparison of this empirical curve with a Gaussian curve, when brought to the same scale, gives results of important biological value. Similar calculations for the gastrula of sea-urchin showed that the frequency of deviations of less than 0.5 (in the range of 0 ± 0.34) is greater than the theoretical Gaussian value. With an increase in the magnitude of the deviation its observed frequency approximates the theoretical value. Stated statistically: the distribution of mitoses relative to the axis of "the whole" is, at the stage of gastrula, clearly subnormal, i.e., the symmetry in the distribution of simultaneous mitoses, relative to the axis of "the whole" is higher than if the mitoses were distributed by sheer chance. Different results were obtained with other materials: onion root, crystalline lens and cornea. Here the empirical curve practically coincided with the Gaussian curve. The distribution of mitoses in these cases is normal.

What is the biological meaning of the factor of subnormal distribution of mitoses?

In the case of normal distribution, the onset of mitosis is a random event which relates to its geometrical position in the primordium, but this is not the case when distribution is subnormal. The genesis of either event, whether due to chance or not, can be entirely different. The term "chance," used as a generalized concept, is misleading. Rather, Event A (mitosis) occurs "by chance" in relation to Event B (the position of the mitosis). This can be interpreted in the following way: A is not a function of the variable B only, but depends also on some other variables C, D, . . . , which are not closely related to B, i.e., which vary independently of B. If A is nonaccidental in relation to B and has subnormal distribution /1/, then the factors determining A are related to B, i.e., vary as a function of B, although they may still have a certain degree of freedom. Through B, these factors are also related among themselves. The pattern of distribution of the elementary processes in relation to "the whole" makes it possible to draw conclusions about the way a given process is determined. The subnormal distribution is equivalent to the degree of interrelationship of all the factors which determine the process. This pattern is defined by Gurwitsch as determination of conditioned events. Normal distribution implies independence of, at least, one of the determinant factors. Determination of an event by a complex of factors is defined by Gurwitsch as rate-setting. The most important conclusion to be drawn from his observations is that a regular, geometrical morphogenesis is also possible under conditions of rate-setting solely of the elementary morphogenetic processes. "The whole" in this instance acts as a rate-setting factor which appears at random (i.e., depending also upon other factors which are not related functionally to "the whole").

These observations all indicate that the concept of "the whole" can be applied without any experimental intervention even to the commonest forms, (which appear not to be regulatory systems) by using data taken from a natural process, e.g., distribution of mitoses in the meristem. It thus became possible to obtain results which are not achievable by the experimental-causal method, and which lay the foundations for new investigations. The discovery of rate-setting connections logically caused further research to follow two separate paths: one led to research on mitotic factors (unrelated to "the whole")which brought about the discovery of mitogenetic rays. The other line of investigation continued to decipher the ways of action of "the whole," which, as we have seen, are far from clear. At this early stage, it was already obvious that random cell division, relative to "the whole," undermines the strictly deterministic concepts of the mechanism of heredity, because a natural, heredity-determined morphogenesis is possible and, in most cases, realizable without precise indication of the time and place of each separate mitoses.[1*] This points to the nonsegregative feature of hereditary factors, the next matter for discussion.

The problem of realization of hereditary characteristics

The discovery of Mendel's laws and the publication of several brilliant works in the field of cytology brought about the first breakthrough in chromosomal genetics. At that time, Gurwitsch was one of the few who saw that Mendel's theory cannot, by its very nature, encompass the whole problem of heredity. Gurwitsch's point of view was the natural viewpoint of the embryologist, interested primarily in understanding the process of realization of hereditary characteristics in embryogenesis. He wondered about the fact that "embryogenesis, the process of formation of the organism, falls strangely outside what is known of heredity. The impression is created," he wrote, "that the transmission of hereditary characteristics is a special . . . process taking place alongside embryogenesis, the study of which can be performed independently" /1, p. 459/. The final conviction which never left Gurwitsch was that Mendelism in all its forms, and especially in its study of genes, is inapplicable and absurd as a general theory of heredity. This was supported by the following considerations. Formal genetics operates with traits (which segregate during hybridization) of a most variable nature: chemical, cytological, macromorphological, etc. It is accepted that the entire organism can be divided into such Mendelian traits. However, every embryologically-minded scientist will realize immediately that such a statement is unacceptable. Characteristics which can be subjected to genetic analysis usually represent the final result of prolonged processes of development; however, the developmental considerations are not usually taken into account in the study of genetics. Genetics is interested in the mechanism of determination of, let us say, the color or the size of the eye or the hair, while those basic general morphogenetic processes, as a result of which the eye or the hair is formed, and without which the genetic factor would be deprived of any field of action, are beyond the range of its interests. But where is the logical justification for making such a sharp distinction between the "embryological" and "genetic" stages?

Several investigations and theories have appeared during the last decades which attempt to decipher the process of realization, and which are based on formal genetic principles (Goldschmidt,[2] Waddington[3]). But do they answer Gurwitsch's criticism of Mendelism? Apparently not, because, by definition, the concept of the gene cannot explain the processes of realization of species heredity taking place in the embryo.

---

*[For this and other references appearing in this paper, see "Notes" at the end of this paper.]

Gurwitsch wrote: "Given a body which is characterized solely by chemical parameters, we can, at least in a general way, draw the boundaries of its possibilities, and thereby reveal its contradictions by conceptualizing a connection" (between gene and trait – L. B.)

Let us see what the formulation of the gene concept irrevocably implies and what it leaves open.

The gene does not claim to determine fate, i. e., the course of evolution of the cell containing it. But if, in the sequence of events in which the cell is involved, certain parameters preserve some degree of freedom, so to speak, then the gene chooses one of the possible variants.

In this form, the relation of genes to chromatin does not meet any obvious contradictions.

However, an examination of the contents of almost all genes (with the exception, perhaps, of the genes of pigments), persuades that their formulation is a collective concept, i.e., it cannot be expressed in the language of the individual cell. The attempt to localize the genes within the cell is, therefore, self-contradictory, as seen in one of the clearest examples — the leaves of nettle [Urtica] with the Mendelian variants of serrate and smooth forms. Realization of a certain variant is conceivable only as a result of different distribution of the foci of predominant multiplication or extension among the homogeneous cells of the leaf. The principle of distribution in itself has a purely topographical character, i.e., it can be described in a certain system of coordinates relative to the primordium as a whole, or relative to certain axes, but certainly not to any one cell.

Thus, the designation, "the gene of serrate or smooth trait" is itself senseless, insofar as individual cells are concerned.

This contradiction is even more prominent in the case of many morphological Mendelian traits. The form (or configuration) is realized out of many elements by only one of two pathways: by simple scheduling of the entire "mosaic" or parameters of behavior in every cell (determination), or by establishing certain "orientors" (landmarks), in the achievement of which, the cells are endowed with a considerable degree of freedom (rate-setting).

In both cases, the spatial direction forms one of the parameters of cellular behavior postulated by the gene. This is somehow determined by the position of the given cell within "the whole," i. e., in the complex of other cells. If we accept that the totality of cells has a directing influence on the individual cells, then the list of postulates attributed to the genes includes, not only their influences on intracellular processes, but also on extracellular processes. This requirement becomes compatible with the chemical nature of genes, if we think of gene action in chemical terms, for instance, the diffusion of chromatin from the cell into the surrounding environment. However, the postulated action is undoubtedly of a vectorial nature and merely this fact forces us to reject the commonly accepted definition of the gene.

It stands to reason that the relation between genes and chromatin is not the main problem, although an essential and even decisive role is attributed to it in genetics. The difficulties and contradictions would not be removed even if, as they sometimes do, the partisans of hereditary characteristics would also recognize, apart from the chromatin, the so-called protoplasm [cytoplasm] or some of its special elements.

The radical contradiction lies in the attempt to segregate the Mendelian characteristics, which, are in fact, (apart from the pigments) nonsegregative. This is preformism in its clearest form; not only are the revolutionary concepts of equipotentiality and equifinality consciously ignored, but principles of action are formulated without any indication as to the course of their realization /7, pp. 169-171).[4]

Thus, formal genetics cannot form a basis for a general theory of heredity, and it is logical to consider only finished characteristics as the subject of this theory. As Gurwitsch writes, "with the same justification, we could consider as a subject of heredity any other transient stage of the embryonal development. Logically, we must recognize the process of formation in typical embryonal development as a proper subject of heredity, irrespective of any stage."

This formulation of the subject of heredity leads to a radical change in our thinking on the nature of "hereditary factors," as these do not determine a certain special condition but, rather, induce a process which changes conditions. And further on: "We have to regard from a new standpoint those processes, which represent the changes or movements of a certain substrate as a function of certain factors" /10, p. 858/. These unified factors of heredity-development should not be regarded as separate entities but as equations of movement of the embryonal elements. "When talking about the causal explanation of the appearance or change of movements," wrote Gurwitsch, "we, first of all, have to solve the problem of the forces causing the observed process. It is still better, of course, if we can also find out about the origin of these forces but, as a matter of fact, knowledge of only the latter is useless. This is, essentially, a dynamic viewpoint and, by its nature, our investigation can be defined as a dynamic theory of heredity" /10, p. 858/. However, "it should be kept in mind, first of all, that we are not interested in the change or movement itself, or in the magnitude of the performed work, or in a certain form of energy, but mainly in the specificity of the process.

"The specificity of the change or movement of a certain body is, however, not determined by a specific form of energy attributed to it, but by the location, direction, intensity and duration of action of all the forces and similar 'conditions of the system' characteristic of a given combination.

"The object of our interest is not the forces, but the sum of these conditions of the system" /10, p. 859/.[5]

Clearly, this approach is easiest to carry out with processes like the displacement of cellular elements or the specific distribution of mitoses in homogeneous meristems.

The concept of trajectory can be applied only in the figurative sense to processes such as cellular differentiation; however, this field has real developmental prospects along the lines indicated.

We shall now discuss the problems of synthesis in embryology, i.e., creation of the general laws which describe the "conditions of the system" for fairly long periods of individual development.

The demand for laws describing sufficiently long periods of development

"If we concentrate on a sufficiently defined, new, 'unexplained' phenomenon," wrote Gurwitsch, "then the explanation frequently employs a comparison with a larger or smaller class of well-known phenomena. For example, the mechanism of invagination of the embryonal leaf is explained by differences in pressure on its two sides, by ameboidal movement of the cells, etc. The process which interests us can be explained as long as we do not ask why the difference in pressure occurs or what the mechanism of the ameboidal process is, etc.

"But the inquiry and the formulation of the problem itself are senseless if we deal with a prolonged, uninterrupted sequence of phenomena and not with a specific phenomenon. Let us presume, for a while, that a separate explanation can be given for the mechanism of each element in a sequence A, B, C, . . . For example, process A is swelling; process B, a chemical reaction, etc. However, it is less interesting to consider a single phenomenon than to consider the problem of why such a regular pattern exists at all in the sequence of apparently dissimilar processes. An additional problem is the selection of certain discrete processes A, B from a continuous sequence of events.

"In biology we are faced mainly with this kind of problem, and embryogenesis itself is essentially such a problem. Here we must look for a special kind of explanation.

". . . A process becomes conceivable only when we can create — instead of a purely phenomenological multitude and a variegated alternation of occurrences — a picture which, although arbitrarily created, is less variegated and closer to reality.

"In general lines, the actual task can be set forth as follows: it should be possible to break down the entire, analyzed cycle of events into a not-too-large number of stages, each representable as a monotonic function of certain predetermined conditions with a single variable, which can be conceived as time, the traversed path, etc.

". . . Even a partial result in this type of analysis of the evolutionary process (embryological — L. B.) undoubtedly means a step forward" /6, pp. 8–9/.

It is obvious that this viewpoint is incompatible with the methodology of experimental embryology, especially modern experimental embryology. The problem of the natural sequence of processes (in time), of the "irrepressibility of embryogenesis" (favorite expression of Gurwitsch) is not raised at all in the usual representation of the mechanics of development, from which these considerations are apparently deliberately omitted as a matter of principle. It is assumed that for each A → B transition (taken at an arbitrarily chosen stage of development). There is a specific "factor of transition," while none of the components has to be identical with any component of the B → C transition factor, or of any other factor. Consciously or subconsciously, the experimental embryologist assumes the existence of transition factors in a number equal to that of the transition steps, and many modern scientists (for example, E. Wolff,[6] Grobstein[7]) make it their task to write down a list of such factors. This approach would be more understandable if the embryonal development were a chain of equal transitional steps, well-determined and always on the same level. But if one observes the regulations operating at every possible level, the rate-setting, and the undetermined relationships among various levels, then the above task appears senseless and futile. Finally, this type of analysis (like that of formal genetics) overlooks the fact that the results of morphogenesis, expressed in spatial and vectorial magnitudes, cannot be deduced from transition factors, which are usually defined chemically. It is this sort of uneconomical investigation which has led to a general situation prevailing in the mechanics of development "where the experimental results allow no extrapolation beyond the given experiment."[8]

But is another approach possible at our present stage of knowledge in embryology? Are there sufficient facts, and is experimental analysis sufficiently elaborated to attempt synthesis?

In the opinion of Gurwitsch, analysis has gone considerably beyond reasonable limits.

"It seems to us that the impulse to analyze manifests itself either in a genuine spirit of inquiry or in mental laziness. The spirit of inquiry is genuine only when the analysis is not an end in itself, but a first step and necessary premise for a conscious and rational new development. The analysis undertaken in blind imitation of a project set up under different conditions for a different purpose betrays mental laziness . . .

"It is therefore imperative . . to draw a line between those results of analysis which have cognitive value and those which do not . . . Let us ask a question which may seem naive at first: 'Do we have to know everything about the object of our observation?' The usual answer will be affirmative but unrealistic, as shown by another question: 'Why do we have to know this?' Most modern authors will probably answer: 'It is interesting because it is unknown. But, as a matter of fact, the answer should be: 'I could not think of any other subject.'

"And so, it obviously becomes necessary to make a logical selection among the innumerable possibilities of analysis . . .

"The biologist is guided in his analysis by instinct and, also, by his belief (perhaps questionable) in biological complexity . . . The layman sees scientific accomplishments as very complete and does not realize that great discoveries come from the application of essentially simple principles.

"When we speculate about life processes we are faced with two alternatives: either an infinite complexity or the comparative simplicity of nature.

"The sage investigator chooses the right alternative at the right time and avoids the extreme and the one-sided approach.

"The analytic method of studying life processes should be regarded as one of the approaches to the understanding of biological problems and should not be an end in itself" /7, pp. 67–71/.

These are the basic assumptions in the Gurwitsch critique. The purely deductive results of his analysis laid the foundation for the elaboration of an entirely new concept of a more inductive nature. However, it should be stressed that Gurwitsch advanced this concept only when he was fairly convinced of the inadequacy of the illogical and contradictory theoretical assumptions available in his time. Gurwitsch saw the only resolution of these contradictions in a "nonclassical" notion which is examined below.

2. SUPRACELLULAR EMBRYONAL FIELDS AS FORMAL INVARIANTS OF DEVELOPMENT

General criteria of reliability in the "field principle"

The first and main task was to find evidence of certain "conditions of the system," related to "the whole," and invariably present in the embryo during sufficiently long periods of development to determine those aspects (parameters) of elemental behavior of interest to us.

Let us say, for example, that the series of elements (cells) a_1, a_2, $a_3 \ldots a_n$ can be compared by means of P which can take the values p_1, p_2, $p_3 \ldots p_n$. In the beginning of the process of development, the elements are indifferent (undetermined) with regard to P. After some time element a_1 receives the value p_1; element a_2, the value p_2, etc. This is a process of natural differentiation of elements with regard to character P. A priori two types of such differentiation are conceivable:

(I) In certain elements there is a hidden predisposition for given values of P; the final differentiation is the consequence of specific preliminary differences between elements, which are not equipotential with regard to P.

(II) The elements are equipotential, i. e., have equal possibilities of acquiring any of the values $p_1 \ldots p_n$, but they are situated in the field of action of certain external factors which are common to the whole embryo and are relatively invariable. Acquisition of p_m value by element a_m depends on the coordinates of a_m in this field. This is the most general formulation of the "field principle" in biology.[9]

The "field principle" depends on finding criteria in the behavior of elements to support one of the two statements.[10] Gurwitsch deals with this problem in his paper "Heredity as a process of realization" /1/, as he does in one form or another in many of his other papers published between 1910 and 1930. Due to lack of space, we shall examine here only his final conclusions.

There is no absolute boundary between the two statements, but a fairly reliable conclusion can be reached concerning the likelihood of (I) or (II). The following interdependent principles of selection between (I) and (II) are suggested. The statement that the elements are equipotential and that their fate is determined by an external field is more likely than a statement on the nonequipotentiality of elements under the conditions described below:

1. If we assume that the primordium can be divided into a number of zones (or areas) related to its axes in the simplest way (usually symmetrical with regard to its axes or center), we find a specific zonal distribution of the P variants and the relationship between the coordinates of a given zone and its P variants can be illustrated by a fairly simple curve.
2. The P variants are distributed within each zone according to a fairly simple pattern.
3. Following consecutive shifts of certain elements, the order of their mutual disposition increases gradually so that, for example, the contours of a later primordium can be expressed by a simpler analytical curve than the contours of an earlier primordium.
4. "The whole" is more accurate than its parts ("the enclosing is more accurate than the enclosed"). "Accuracy" applies here to both location and timing: in the first case, it stands to reason that the contours of "the whole"

are mathematically more accurate than the shape and disposition of any of its parts; in the second case, the final result is regarded as the unification of two or more component processes. While their relative rates may differ largely from case to case, the final result is maintained constant. In most instances, "accuracy" can be envisaged in both its aspects at the same time.

Regardless of the fulfillment of these conditions, it is necessary to show that the "law of the invariant" is valid over a fairly long period of development.[11]

The rest of this chapter will examine several works by Gurwitsch and other authors who argue in favor of the "field viewpoint" in the determination of the behavior of elements in a series of plants and animals. We shall examine these works in chronological order and shall discuss the advantages and drawbacks of this preliminary form of the "field theory," its evaluation and the response it evoked.

"Mechanism of hereditary transmission of the form" /2/

Gurwitsch's main objective in this work was to isolate long periods of development during which the behavior of cells could be described as an invariant function with a single progressively increasing independent variable (time or path). The materials for study ranged from the central nervous system (mainly brain vesicles) of shark to those of chicken embryos.

Any embryologist will agree that these materials are eminently suitable for the purpose. The epithelial wall of the neural duct, and of the brain vesicles which develop from it, maintain over a long period of development an almost uniform structure, without noticeable cytodifferentiation; the observable changes are invaginations, evaginations and inflections, i.e., purely kinematic processes. These are easily explained in terms of the trajectory and dynamic conditions of the system. Morphogenesis of brain vesicles, however, is not a simple one-way progressive process of directed growth. It is a complex of invaginations, evaginations, shiftings, thickenings — in short, a process of remodelling. We still lack a single functional expression for this sequence of different processes. It would be very helpful if a term could be found, expressing a general point of view, rather than a limited application to each morphogenetic process and its cause.

The generalizations were preceded by thorough cytological investigation of the cellular processes to which inflection and thickening of a layer are related, and how these changes are connected with the distribution of mitoses and cell migration. It was found that a close connection exists between the intensity of cellular reproduction and subsequent morphogenesis; at the stage preceding evagination of certain parts, the number of mitoses in these parts increases markedly as compared to neighboring zones.

Another subject of investigation was the relation between the inflection of a flat part and the change in cell density in the concave and convex parts following the evagination of a previously flat surface. Let us assume that cell density decreases on the convex side, while it increases proportionally (due to the decrease in the radius of curvature) on the concave side. Then, such an inflection can be regarded as "passive," as it is due to forces external to the given part, while the part itself behaves like a flexible hard pivot with compressible contents (like any bent hard object). If, on the other hand, the convex side maintains an equal cell density to that on the concave side, this implies that there is a more intensive "flow" of cells from the zone of multiplication to the external side than in the nonbending layer. The zone of cell multiplication is situated in the neural epithelium in the internal layer. If the cell migration from the internal to the external layers could be convincingly demonstrated and quantitatively related to the inflection of the layer, it would support the idea of an "active"type of inflection.

It was found that both passive and active inflections occur in the process of morphogenesis of the brain vesicles, which means that it is senseless to speak about any specific and invariable aggregational properties of the cellular layer. However, active inflections which can be recognized by the lack of change in cell density on the external side, were more frequently observed. Consequently, the inflection is associated mainly with a change in the "rate of exit" of cells from the zone of cell multiplication. The accuracy of this relationship is indicated by the fact that cell migration from the zone of cell division does not lead to any visible thickening (or thinning). In most cases, "the migration of cells occurs according to the size of the available space" /2, p. 533/.

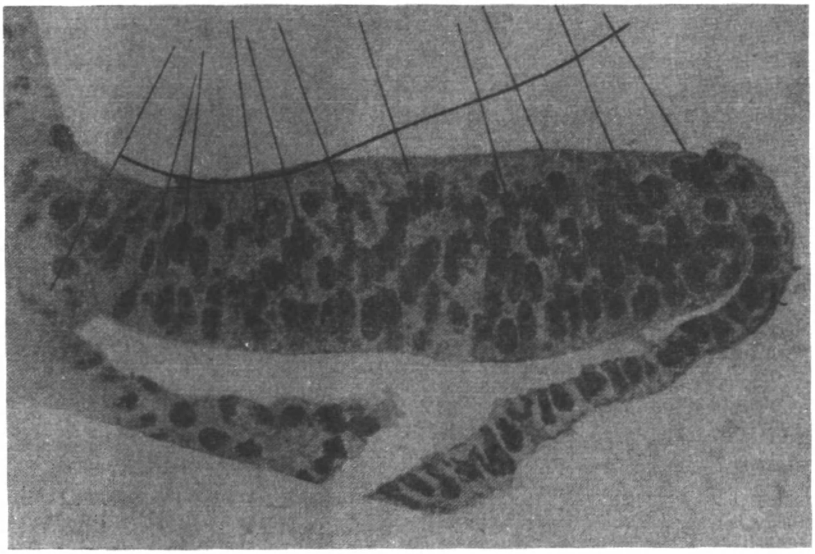


FIGURE 1. Prognostic value of the orientation of nuclear axes in the mitotic zone of the neural tube and brain vesicles in Scillium

Diagram represents the contour of the subsequent stage, based on the inclination of nuclei in the wall of the neural plate /2/

The absence of change in cell density from stage to stage, refutes the assumption that inflections of the layer are related to a specific distribution of cells (thickening and thinning) which tends to "stretch" certain regions and compress others. Other parameters had to be found with which to express the changes in form, such as the angles of inclination of cellular (nuclear) axes in the zones of cell division, relative to a line perpendicular to the given region of the layer. As a rule, the long axes of cell nuclei in the zone of cell division form an angle of up to 20° with the perpendicular to the surface of the given region. But even more interesting is the fact that the curve perpendicular to the cellular axes in the zone of cell division is surprisingly coincident with the contour of the subsequent stage of development of the given region (Figures 1, 2). If the given region is going to thicken, the nuclear axes in the zone of cell division have a smaller inclination than in the external layer; if, on the contrary, the curvature of the region will increase, then the divergence of the axes in the zone of cell division exceeds the divergence of axes in the external layer. This coincidence of a theoretical configuration with the observed one is not haphazard, as it has been observed in many regions and at many stages of development. On the basis of this coincidence, it may be said that the orientation of nuclear axes in the zone of cell division has prognostic value. The author figuratively makes the comparison with a rudder, the turn of which precedes the turn of the whole ship.

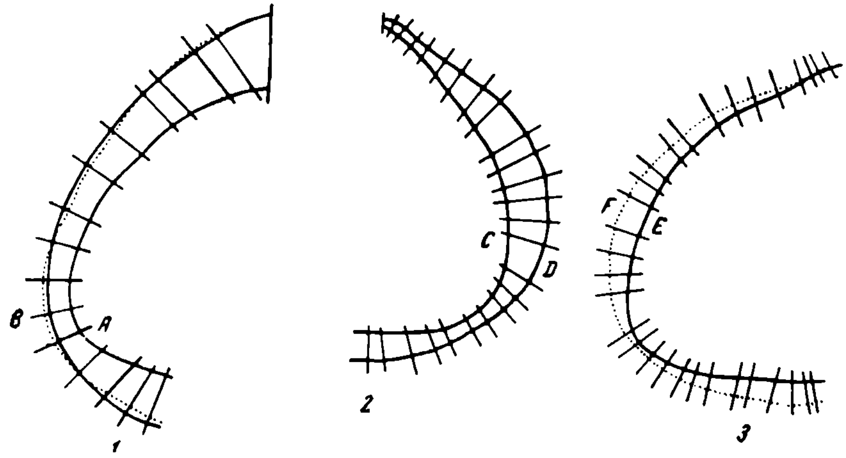


FIGURE 2. Reconstruction of configurations corresponding to stages B, D, F, on the basis of the preceding stages A, C and E
One can see coincidence of the forecasted configurations with the observed ones. In case 1, the real configuration is illustrated by a dotted line; it is somewhat less accurate than the forecasted configuration 2 .

The analysis showed that even such an elementary morphogenetic process as inflection of the epithelial layer requires coordination of heterogenous cellular processes: cell division, migration of cells from the zone of cell division into the external zone and a specific inclination of nuclear axes. It is clear that all these have to be precisely coordinated in time: for example, the larger the divergence of the axes, the higher the "rate of exit" of cells from the zone of cell division in the subsequent stage, and the higher the mitotic coefficient.

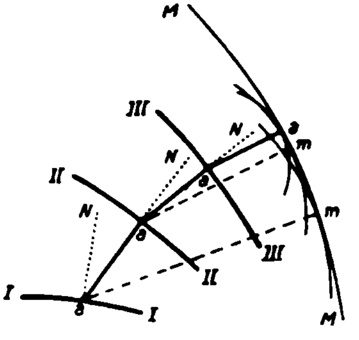


FIGURE 3. Illustration of the trajectory of a cell from an epithelial layer which is under the influence of DPM (surface MM)

I. II. III — contours of consecutive stages of the epithelial layer; N — perpendicular to the surface of the layer at any moment; am — shortest distance from the given point to the surface of DPM. The true trajectory of the cell (nucleus): aaaa — the bisectrix between the two lines /2/.

It is awkward to attempt to express morphogenesis of the brain layer as a sum of the life cycles of separate cells, determined strictly and in detail (i. e., interrelation of morphogenesis from a "mosaic, " cellular-determinist point of view) in view of the above heterogeneity of interrelated elementary processes. Moreover, at this point, it had become completely clear to Gurwitsch that the first link in the life cycle of the cell, mitosis, is only regulated, but not determined in time — the cell "enters the game" at a chance moment. Strict determination in time of the rest of the cellular life cycle would bring chaos to morphogenesis rather than order. The author takes a position opposite to the cellular-deterministic one: he adopts the "field viewpoint"; and he postulates the presence — in the space around the rudiment — of a certain dynamic surface. This surface corresponds in its disposition to the definite configuration of the rudiment, as if "preforming" the latter "dynamically." Hence the term "dynamically preformed morph" (DPM), to designate the power-exerting surface. The nature of its action on the elements is precisely determined: it orients and attracts the cells of a layer in such a manner that the axis of each cell tends to become perpendicular to the nearest point in the DPM. The actual orientation of the cellular axis at any moment coincides with the resultant — (bisectrix of the angle) — between the perpendiculars to the layer at that moment and at the nearest point in DPM. As seen in Figure 3, the arbitrarily chosen cell 'a' follows a curvilinear path in its movement toward DPM and never intersects the surface of the layer at a straight angle. The concept of DPM is, therefore, a brief and general formulation of the divergence between the configuration of the layer and the orientation of the cells, which seem to "run" one stage forward. However, DPM is demonstrably not a tautology. It enables us to make quite unexpected and clear forecasts which cannot be made from any other viewpoint. Let us compare two examples: 1) The shortest distance between a given point of the layer and DPM is strikingly different from the other distances (Figure 4, A — aa_1); 2) two minimal distances between the given point and DPM are slightly different from one another (Figure 4, A — cc_1 and cc_2). Both cases can be found in the lateral and apical parts of the convexity abc of the brain vesicle, which increases more in curvature as it approaches the final configuration $a_1b_1c_1a_2b_2c_2$, which has been denoted as the DPM. If we accept the idea of the "attracting-orienting" action of DPM, then, in the first case, we would expect orientation of the cellular axes strictly according to the direction aa_1 (oblique to the layer) with insignificant deviations from this direction. (The effect of DPM is strong and markedly polarized.) In the second case, considerable divergence is to be expected in the direction of cellular axes around the central, zone position (perpendicular to the layer) insofar as the effect of DPM is weakened (slightly polarized)

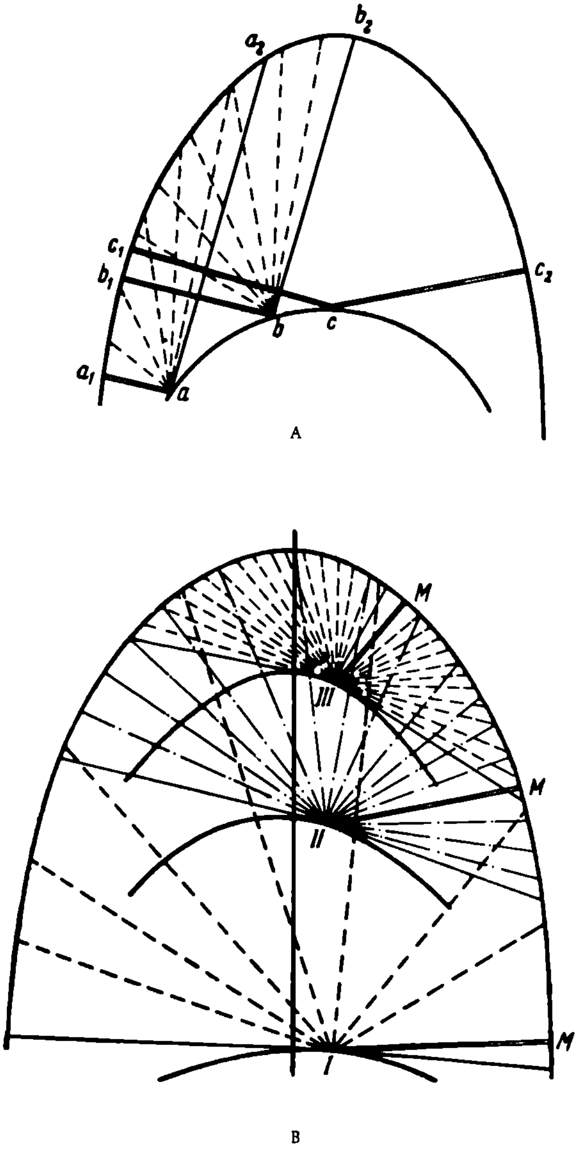


FIGURE 4. Diagram of action of the U-shaped DPM ($a_1b_1c_1a_2b_2c_2$) on various points of the layer surface (abc)

A — point a has one clear direction of maximal action (a_1a); point c has two almost opposite directions (cc_1 and cc_2); B — as the layer approaches DPM, a certain point (I-II-III) acquires one clear direction of maximal action (M-III is much shorter than all the other distances between point III and DPM; the opposite is true for point I). The directions of maximal action (shortest distances) are indicated by double lines /2/.

and originates from opposite sides. In later stages, as the layer approaches DPM, a clear minimal distance is again observable between some point close to the center and the morph (Figure 4, B — I-II-III); dispersion of the axes should decrease in comparison with its previous values.

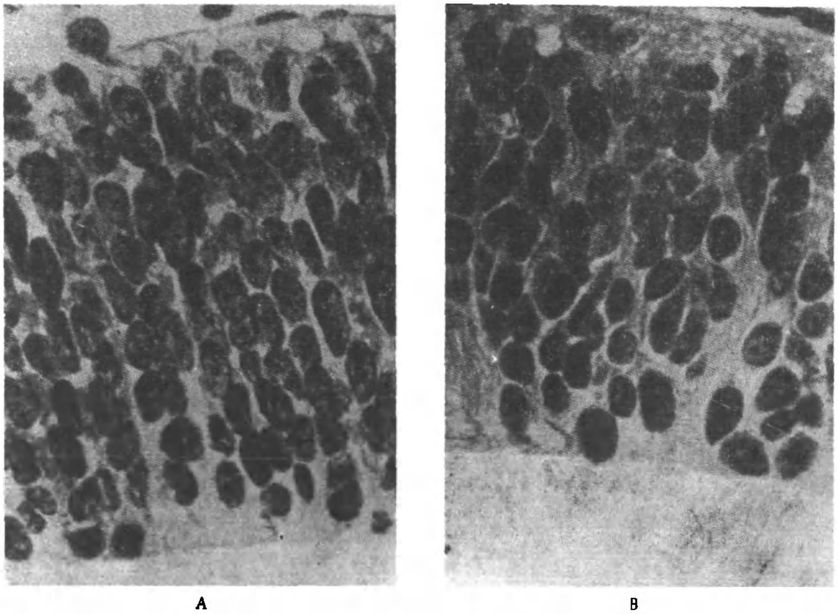


FIGURE 5. Two areas from the wall of the brain vesicle, corresponding to Figure 4A

A — area with clear maximum (a-a_1), minimal dispersion of nuclear axes and obliquely situated nuclei, which are oriented according to the direction of minimal distance to DPM; B — area without clear maximum (c-c_1-c_2); with maximal dispersion of nuclei / 2/.

All these are, of course, nontautological assumptions, which can be satisfactorily confirmed by the proper calculations, and in some cases, are obvious even without any special calculus (Figure 5). In this way, the most variegated changes in the orientation of nuclei become compatible with an interpretation based on a single, invariant law.

"The concept of the embryonal field" /3/

In this work, Gurwitsch deals with morphogenesis in the following plants: a Compositae, Matricaria chamomilla, and two Hymenomycetae, angio- and gymnocarpic fungi.

The mature inflorescence of Matricaria has the shape of a paraboloid of revolution (Figure 6 — D, E, F). In the course of development, the contour of the growing inflorescence gradually approaches the accurate parabolic shape (Figure 6, A, B, C). First the top of the inflorescence, and then gradually the basal flowers draw as far as the rounded parabola. Obviously, the lower flowers have to grow faster than the top ones. The peripheral limit reached by a growing flower is a function of two components: growth of the flower itself, and growth of the floral disk. It is particularly important that none of these components, taken separately, has a clearly defined increase in size. In some cases, the floral disk grows more

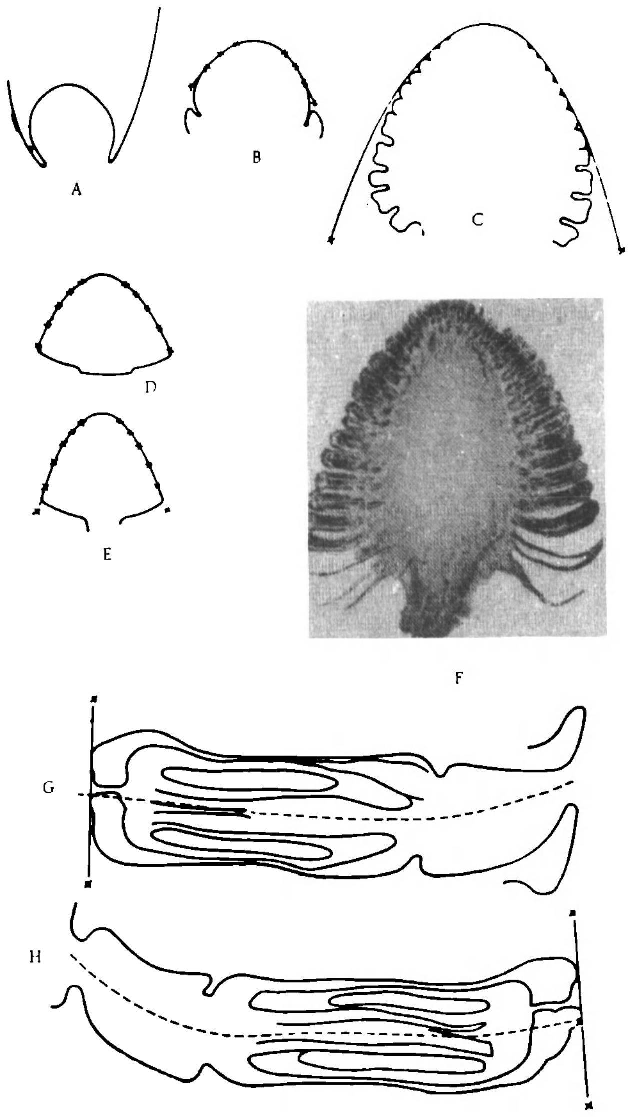


FIGURE 6. Morphogenesis of the inflorescence of Matricaria chamomilla (vertical sections)

A — very early stage (30×); B, C — somewhat later stage with partially parabolic contour; D, E — mature inflorescence (5×) showing complete coincidence with a parabola which is denoted by crosses; F — sagittal section through a mature inflorescence; G — diagram of a left basal flower of normal shape; H — diagram of a right-hand compensatorily rounded basal flower. Both "draw" as far as the rounded parabola, a fragment of which is visible in both drawings (crosses) /8/.

intensively than in others, but accurate calculations show that the center of the surface of each flower continues to move along a linear trajectory which unites it with the center of the inflorescence base, and this is how the accurate parabola shape of the whole is maintained. Individual variations in the growth rate of the inflorescence may even lead to compensatory curvatures of the floral axis and to the stretching of its separate parts (Figure 6, G, H), which again helps to maintain the parabolic shape of the whole.

Gurwitsch assumes that the inflorescence is surrounded by a symmetrical field with a parabolic tube of force, which attracts the surface of the elements (flowers) along raylike trajectories. These unite a given point of the parabola with the center of the inflorescence base, so that the direction and size of floral growth are a function of the position of the flower in this field. While it determines the final limit of growth, the field does not fix rigidly, but only "regulates" (within the limits of the parabolic surface) the behavior of both components of the inflorescence — the flower and the inflorescence.

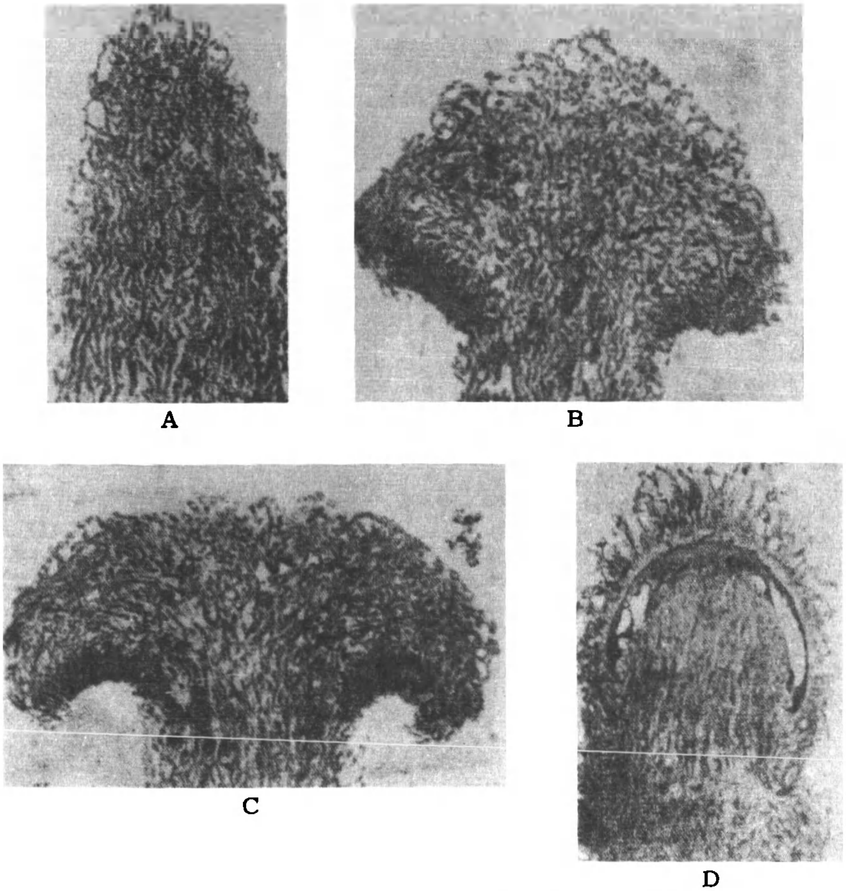


FIGURE 7. Morphogenesis of Marasmius sp. and of an angiocarp fungus of unidentified species

A, B, C — consecutive stages of development of Marasmius; D — formation of a semicircular boundary line in a rudiment of angiocarp fungus. The upper hyphal endings will be torn away /8/.

An explanation of the mathematically accurate shape from a segregative-deterministic viewpoint, by attributing to each flower a predetermined growth rate and by excluding any "universal" law is more difficult as the mathematical law is simpler and the number of elements (flowers) is greater. If it were possible to draw a curve through the surface of, let us say, an "n" number of flowers, which would be no less than of (n-1) degree, such a curve would have a purely descriptive value, as a curve can always be drawn through any "n" points arbitrarily situated. In this case the law is expressed in only a second-degree curve, and it would be senseless to speak of it as a regulating law.

In the discussion above, we have not considered the observed variability in the behavior of each of the two components of the inflorescence, which in combination produce an unchanged result. This fact is clearly incompatible with "segregative-deterministic" concepts.

The data on the morphogenesis of fungi are very simple (Figure 7). The development of the fruit body in the gymnocarp Marasmius sp. begins with a rise from the substrate of an irregular bundle of mixed hyphae; the contours of the young pileus are indistinct. With further growth, its contours become more accurate and smooth, and the hyphal ends become oriented in parallel lines (Figure 7, A, B, C).

Analogous processes showing gradually increasing distinctness appear in a more interesting and instructive form in the angiocarp fungus (unidentified species). In the beginning, as in Marasmius, the contours of the apex are indistinct. Later on, somewhat below the upper hyphal ends, a very accurate semicircular line becomes visible which delineates the contours of the future crown; above this line the hyphal endings become friable and fall off (Figure 7, C). This is a characteristic example of lack of predetermination in the growth boundaries of separate elements (in this case, of hyphae); their growth is merely regulated and its accuracy is introduced into the system secondarily in a very simple and radical way — by removal of the superfluous material.

"Application of the field principle to analysis of the processes of embryonal differentiation" /12/

This work by Felicine-Gurwitsch is one of the few which point out the possibility of applying the "field principle" to a process of cytodifferentiation. In this process certain differentiated cells separate out from the primarily homogeneous mass and form a spatial or two-dimensional "cellular net." If seriously considered, this apparently modest work can form the starting point for an entirely new approach to cell differentiation. The main problem of the work, which was performed on developing retina of amphibians, is very simple. Can one find certain components in the processes of cytodifferentiation which are functions of coordinates of the differentiating cell in relation to the axis of symmetry of the whole retina?

The investigation compared the histogenesis of receptor cells in central and in peripheral areas of the retina. In amphibians the retina grows for a long time apositionally by addition of new cells from the marginal mitotic zone. Consequently, the central cells are much older than the peripheral ones.

The influence of the coordinates of elements on their histogenesis was visible in the inversion of the processes of growth and differentiation in the peripheral cells in relation to the central cells. While the latter enter the stage of differentiation after having grown to a large size, the former have to undergo differentiation before they grow (Figure 8). Thus, assuming the origin of the coordinates to be the point of exit of the optic nerve, the disposition of cells in the retina is a function of the rate ratio of the two processes taking place in the retina — growth and differentiation. The action of the field may perhaps be illustrated in this way: the relationships between the two processes are a function in time of the coordinates of the given element; there is no compulsory and close connection between the processes themselves, and an identical final result (an identically built receptor cell) can be achieved under conditions of different rates of the two processes.

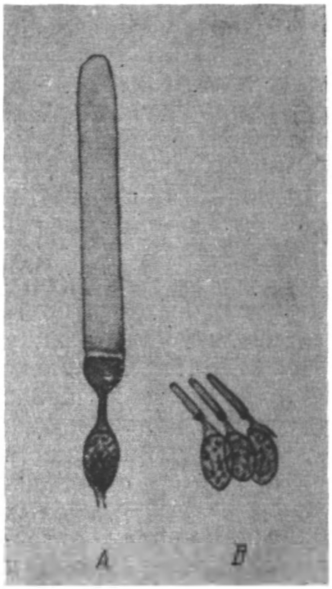


FIGURE 8. Rod receptor cells in the retina of a young frog immediately after metamorphosis

A — from the center of the retina; B — from the periphery, equal magnification. An analogous degree of differentiation was reached with widely varying sizes /8/.

This is the place where Gurwitsch thinks the fruitful collaboration between experimental and analytical embryology comes to light. The experimental scientists have accumulated a large and heterogenous amount of data concerning the segregation of processes which, under normal conditions, are interrelated in time and space. The ability to reproduce experimentally a segregation of this type indicates the mutual autonomy of the processes. Their normal correlation cannot be explained by their direct interrelation, but only by the action on them of a general type of field, which is "situated at a higher level." The relations between such "segregated" processes would not derive from the properties of the processes themselves, but would be "forced" upon them by the field.

This work describes the gradual regulation of histogenetic processes taking place within the boundaries of a single cell on one hand, and of the mutual disposition of rods and cones in the retina, on the other.

"Morphogenic field of the cartilage primordium" /13/

In this work by Anikin, the "field principle" enabled the author to make several accurate quantitative predictions.

The regular rosette-like disposition of nuclei in a cross section through a phalangial rudiment of triton or axolotl suggested the action of a single field of a fairly simple structure. It suggested that the point-like source of an isotropic field, which exerts a repellent force on each point on the surface of the surrounding cellular nuclei, is situated in the geometrical center of the radial rudiment of the cartilage (as seen in cross section). It is accepted that the repellent action of the field source is inversely proportional to the distance of the given point from the field source, i.e., the momentary velocity (V) of the given point in the field is given by the following formula:

$$V = \frac{dR}{dt} = \frac{K}{R},$$

where R — distance of point from the source, K — constant, t — time.

An integration of this equation establishes a relationship between the time of movement of the point in the field and the path traversed during this time. Thus, if, in the beginning, the point was at a distance R from the field source, and then moved centrifugally over a distance r, then the time t' elapsed in crossing the distance r is related to R and r by the following equation:

$$2Kt' = r^2 + 2Rr$$

If we are interested in the instant (simultaneous) positioning of all the points on the nuclear surface (i.e., what we see in the preparation) then we can determine the coordinates of these points ($R+r$ for each point) from their coordinates t' units of elapsed time. That is, we know R for each point, and assume t' = constant, because for each t', R and r are related by an identical equation r^2+2Rr = const (Figure 9).

This law should explain two peculiarities of the cartilage rudiment: 1) the regular variation in the shape of nuclei (from round in the center of the embryo to ovoid and crescent-shaped toward the periphery; and 2) the large gradient between the degree of stretching and bending of nuclei in the periphery of the rudiment and the rounded shape of those lying just a little more peripherally.

The preliminary calculation of forms agreed completely with the observed ones (Figure 10). This is particularly clear in the case of nuclei situated assymmetrically with regard to the field source. The appearance in the periphery of rounded nuclei together with strongly deformed ones can be predicted by justifiably assuming that the deformed nuclei appear as a result of mitosis occurring in the center of the rudiment; these nuclei were for a long time under the action of a strong field, while the round nuclei, situated at the periphery, have never been under the influence of a strong field.

It is remarkable that the regular rosette-like structure disappears during the mitotic bursts, but is restored afterwards as it is visible in another cellular structure (of a later generation). This establishes that the regular pattern of nuclear disposition cannot be explained by their mutual "mosaic" relationships without participation of an invariant regulating factor, as, without this participation, there is no explanation for a restoration of the regular pattern and an addition to another material.

A characteristic of this work is the strictly geometrical interpretation of the field source: the source is visualized as being situated in the center of the rudiment, regardless of whether there is a cell there or not. On the other hand, the "field principle" is presented differently in the works of 1914 and 1922: instead of the earlier attracting "dynamic morph" external to the rudiment, we find later the internal repellent, point-like (and not complex) source. Undoubtedly, one can find the first hints here of a new and simpler interpretation of the invariant, which finally, many years later, culminated in the concept of "cellular fields."

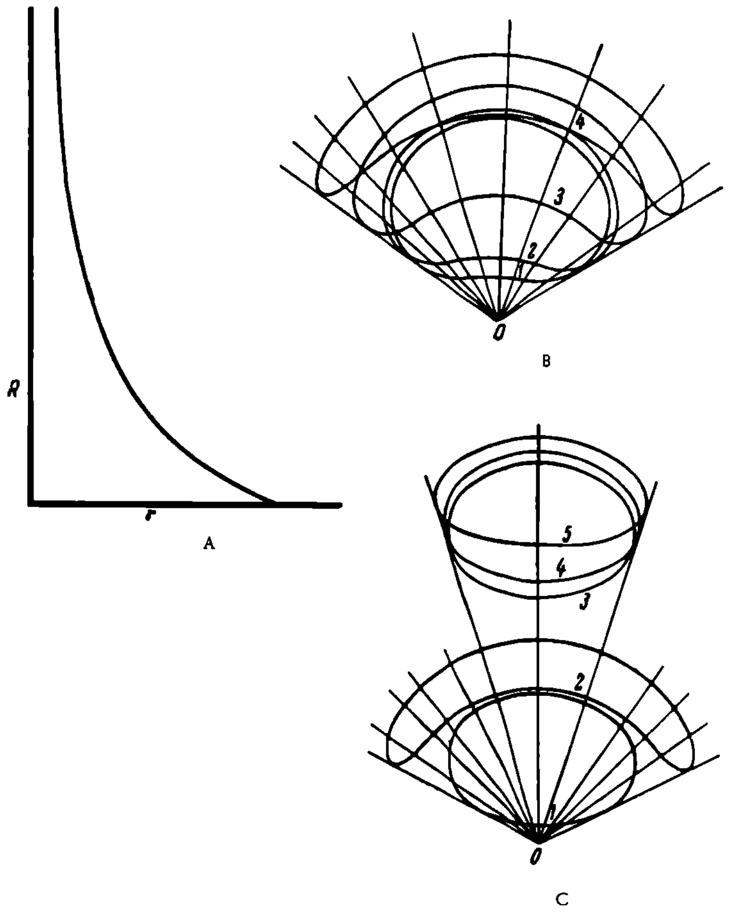


FIGURE 9. Morphogenic field of a cartilage rudiment

A — relationship between the path (r) traversed during time (t) by a certain point on the nuclear surface, and its initial distance (R) from the field source (hyperbola of second degree); B — preliminary calculated changes in the form of the nucleus (1-4) which moves from the field source 0; C — predicted changes in nuclear form as a result of mitosis taking place in the central (1-2) and peripheral (3-5) regions of the field /8/.

Even less lasting was the sophisticated explanation of the complex transformations of spermatids during spermatogenesis by assuming the existence of a point-like revolving field /5/. The same is true for Anikin's explanation of the morphogenesis of the crystalline by means of a geometrical function of a variable field in the concave eye lens /14/.

Strictly speaking, the latter work is part of the theory of cellular fields and it is surprising that the author should completely ignore this theory, (as he ignored the whole "field theory". Instead, he presents assumptions which are improbable from a physical point of view.

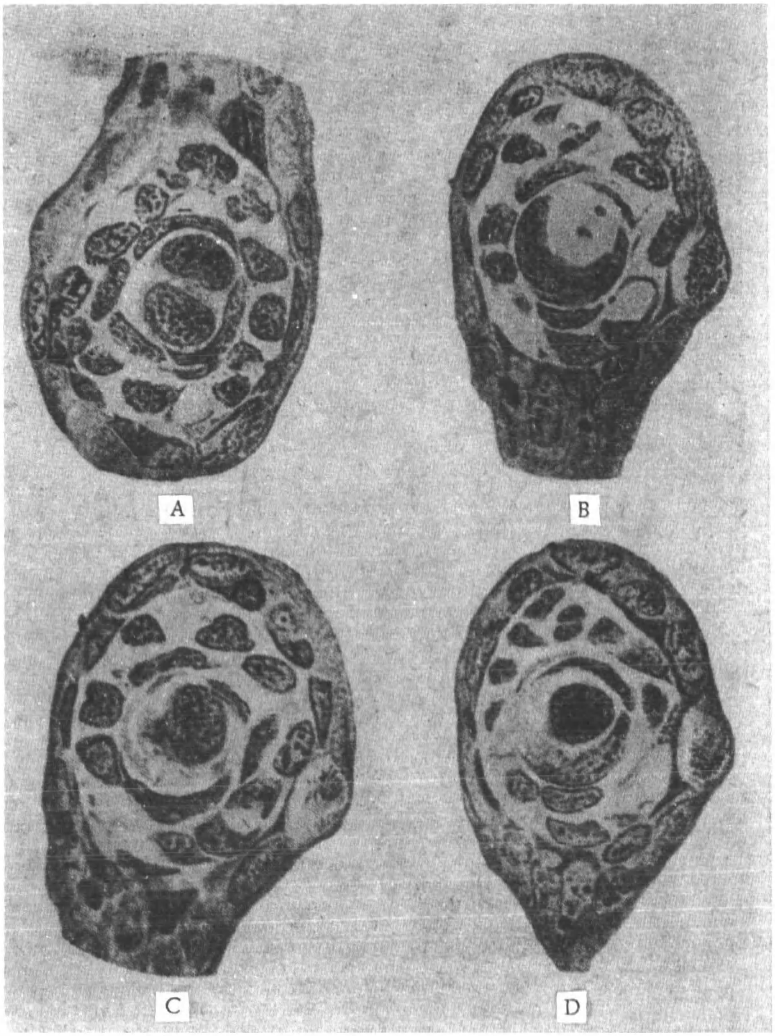


FIGURE 10. Observed forms of nuclei in cross sections through a rudiment of triton phalanx (A-D — four different cases) / 13 /.

Investigations of plant morphogenesis based on the field theory

Two botanical works were published by Smirnov (the first together with Zhelokhovtsev) in which the "field principle" was successfully applied.

The first work /15/ deals with the growth of the leaf of Tropeolum majus. This leaf undergoes, in the course of its development, a complex alternation of configurations: it passes through three, five, and seven-lobate stages, and finally assumes a rounded smooth-edged shape. The authors showed that this sequence of changes (like the development of the leaf under experimentally changed conditions) conforms to an invariant pattern of growth; the contours of the surface correspond to a conchoid type of curve.

In this work, the authors refer to the "field principle" only, as a convenient means of describing a process, but another work of Smirnov /16/ attributes more dynamic properties to the field (stimulating or suppressing growth). It is difficult, however, to draw the boundary between the dynamic and descriptive aspects.

This second work deals with the inflorescence of Coryandrum sativum. The author, using large quantities of material, showed the positive correlation between the length of petals of the peripheral flowers in the inflorescence, and the inclination of the petal axis as it relates to the radial plane of the inflorescence. Such an inclination is often caused by bending of the distal part of the flower stalk. The larger the angle formed by the axis of the given petal with the radial direction, the shorter the petal. The author concludes that the development of petals takes place in the general radial field of the inflorescence corresponding to the petal growth which is situated as close as possible to the radial direction (i. e., which forms the smallest angle with the vector of the field). On the basis of a thorough descriptive and experimental analysis, the author rejects all the explanations of growth suppression of the given petal by means of purely "hydraulic" causes (hindered access of substances due to bending of the flower stalk) or by the direct influence of neighboring flowers.

Recently a short publication by Abel and von Denffer appeared, in which they examine the relationships between the axes of the whole organ and the direction of differential cell divisions in the wall of the leaf blister of Utricularia vulgaris from the point of view of a morphogenetic field /17/. This interesting work shows how wide the applications of the "field principle" are to problems of cytodifferentiation, which seem to be less fitted to this principle than the purely kinematic processes of development.

The above-mentioned investigations conclude the short list of works in which the principle of spatial invariants of development, i. e., of a morphogenic "supracellular field," are used in various constructive forms. These works should be clearly distinguished from that large body of articles and monographs which appeared in the twenties and thirties, in which terms such as biological, inductive, morphogenetic and all sorts of other "fields" were repeatedly and variously used.

In our opinion, none of these works advance the "field principle" beyond the "harmonically-equipotential system" of Driesch. Experiments were set up, and if they demonstrated the equipotentiality of elements in a given area, then the authors were convinced of the presence of a field in this area. However, constatation of the field is nothing more than the direct result of the given experiment, and fails to lead to any predictions or limitations concerning any aspect of the behavior of elements. For example, if an investigator speaks about a field in the rudiment of a limb, of the eye, etc., this does not mean that, in the given kind of experiment, he has proven the equipotentiality of this rudiment within the boundaries of the given area. The term "field" itself has no special sense here, and is, strictly speaking, inappropriate.

The tautology of these field studies brought about some critical reactions, which, with no justification whatsoever, were attributed to the "field principle" as understood by Gurwitsch. We shall examine here only those criticisms directed toward Gurwitsch's work. In brief, they are alike in saying that the field theory explains nothing and that it replaces one obscurity with another, perhaps even more opaque and inaccessible. The authors who criticized Gurwitsch in this manner (for example, L. V. Polezhaev[12]) do not seem to perceive the reduction of a variegated chain of embryogenic events to one fairly simple invariant law as a solution to the problem. This is no tautological replacement of one unknown by another; this is the search for a valid law by the use of essentially new parameters, which are, however, clearly characterized by their action on the elements. Strictly speaking, Gurwitsch's concept can be called "mechanics of development" with greater justification than the trend started by W. Roux, because it explains natural phenomena by the principles of the physico-mathematical approach (physical dynamics) which dates back to Newton. The discovery of invariant laws for long embryogenic intervals is a great success in rational thinking. The deficiencies of the initial formulation of the theory, which were best pointed out by the author himself, were not inherent contradictions. The subsequent history of the theory showed that it has a potential for further development and improvement, which is only possible when the bases are sound.

An obvious deficiency was the absence of a "material basis." Gurwitsch prudently said nothing definite about the source of the field. We did not wish to follow the path of Morganism, which does have a material basis, but shows unsolvable contradictions in the principle of its action on elements. He was also reserved toward Spemann's[13] assumptions, fashionable in his time, which opposed active material structures of the embryo to the passive ones. Finally, Gurwitsch wanted to find invariant laws which would also work under conditions of continuous transformation of the substrate — "in a flow" so to speak. "First an invariance of any price" — this was the figure of speech employed [by Gurwitsch] in the first formulation of the "field theory." As for a solution of the problem of origin and an attainable degree of identification of the invariance based on known facts — that could come later.

Gurwitsch pointed out the following deficiencies in his first formulation of the "field theory":

"First of all, in all cases 'the field' is invariant only for a short period of time. In other words, if a given structure of 'the field' is applicable to the stage of development from A to B, it has to be replaced by a different structure in the stage from B to C, and so on.

"Secondly, 'the field,' as understood by us, is essentially incapable of extrapolation, i.e., we do not see how it can become a general biological principle and find further application in the investigation of later processes of development.

"Finally, the separation of rudiments for which 'the field' is created is, of course, arbitrary and artificial. Only in two cases, hymenomycete fungi and inflorescences of chamomile, where the field refers to the entire organism, was the artificial determination of boundaries avoided.

"The following, however, is the main and most bothersome point:

"The supracellular fields . . . are a comparatively insignificant item of the infinitely complex system of intracellular processes, and remained completely outside the scope of our inquiry. This explains the exclusively morphogenic character of the supracellular fields, in our studies, because they were only useful in those stages of embryogenesis in which we were concerned with the movements of cells or their changes of form."

For these reasons, it became clear to us many years ago that the only valuable idea in our initial conception, the postulate of the invariant, . . . had to be applied in an entirely different approach, and, in all cases, had to be applied to intracellular processes. The actual elaboration of cellular fields appeared inattainable at first and, therefore, the concept did not go further than simple tautology, expressing the idea that cellular fields exist and that they control intracellular processes.

"A completely new situation was created (independent of our main train of thought, only when certain facts connected with mitogenetic irradiation led to the postulate of cellular fields . . . We shall be able to show that the achievements of the former (supracellular fields — L. B.) fit in readily in the latter postulate" (cellular fields — L. B.), /6, pp. 22–23/.

3. CELLULAR FIELDS

The physiological theory of the protoplasm. Molecules as objects of the field action

Gurwitsch, influenced by many, fairly heterogeneous, experimental data, became convinced that the concept of invariant dynamic conditions of the system can be applied, not only on the cellular level, but also on the molucular level of organization. The first results along these lines were obtained by Gurwitsch[14] and other investigators from the centrifugation of eggs of amphibians and of other invertebrates. These experiments showed that accurate morphogenesis and, in any case, cleavage can take place under conditions of very extensive mixing of the ooplasm components, and loss of its visible structure. Therefore, there remains no doubt that "morphogenetic and cytological processes can occur on material other than the normal one; the process of division, for instance, need not take place through the same molecules which participated in the cleavage of the non-centrifuged egg" /18, p. 81/. Thus, the concept of equipotentiality — which does not mean, of course, equivalence in all aspects but only interchangeability of these and other morphogenetic processes — can be applied also at the level of molecular structures of the protoplasm. It follows from these experiments that "even in a coarse microscopic range, one can, in many cases, establish the absence of a relationship between the course and character of the main life processes on one hand, and the 'structure' of material substrates on the other. The term 'structure' is understood here in the most general sense of the distribution of material particles" /19, p. 81/. In other words, there is no single pattern of distribution of molecular structures in the egg, which is the only one compatible with the normal course of morphogenesis (or, at least, compatible with the cellular divisions which immediately followed the centrifugation). Naturally, this empirical conclusion leads to a very general assessment that, with any restorable disturbances of the primary molecular structure, certain purely dynamic conditions remain unchanged in the protoplasm and that they, almost deliberately, restore the disturbed organization in other material. The development of these ideas forms the content of the "physiological theory of protoplasm" /19, 18/.

When it was shown, by using labeled atoms, that continuous intense renewal of the molecular composition also takes place under normal conditions in all cellular structures, without exception, these ideas acquired considerable weight. Gurwitsch called these processes "structured processes." Their stability can be explained, not so much by the constancy of their material composition, as by the supporting influence of external dynamic conditions.

However, these experimental-embryological and biological data can also be interpreted in the following way: the continuity in organization is ensured by the presence of some rigid, stable "framework," which cannot be dislodged even by the centrifugal force, or which is only gradually replaced during metabolism. Experimental facts alone will solve this problem.

Gurwitsch rejects the assumption of the presence of an ooplasmic "framework" which is not influenced by the centrifuge, considering it insubstantial from both factual and logical points of view. Blyakher /20/ expresses a similar opinion, on the basis of personal investigation and several references in the literature. Later investigations apparently also supported this point of view.[15]

Such a general and categoric position cannot be assumed with regard to the stability factors of cytoplasmic structures in other cells; however, this problem can be expressed in accurate physicochemical terms, which specify the means and subject of these still vague "dynamic conditions."

The problem consists of finding out the types of molecular structures encountered in a living system. It is, of course, beyond doubt that there exist various stable molecular associations, connected by covalent, electrostatic or van der Waals bonds. The general characteristic of these molecular associations is that, "in order to break these bonds, some force is necessary, or, what is essentially the same, the structures maintain themselves although they possess a considerable degree of instability (lability)" /18, p. 77/.

In addition to such actual structures, one may visualize, a priori, the existence in the protoplasm of orderly associations of "unconnected molecules, which exist by expending energy, and fall apart as soon as the continuous supply of energy is interrupted. The best known example (model — L. B.) of such a system is the arrangement of elongated molecules in the direction of flow of a rapidly flowing liquid.

"The radical difference between such a molecular 'arrangement' and molecular 'structures' is that, in the case of the latter, energy has to be spent for their disruption, while, in the case of the former, energy has to be spent for their maintenance. The 'structures' are maintained, i. e., they are essentially balanced, while the 'constellations' (as we call complexes of molecules which are not mutually connected) fall apart, and therefore have to be regarded as lacking equilibrium" /18, p. 78/.[16]

The methods by means of which these unstable molecular "constellations" (UMC) can be demonstrated are derived from the definition of UMC given by Gurwitsch: "If it is true that for the maintenance . . . of unstable constellations in the cellular body, energy has to be continuously spent, then the constellations have to possess their own potential energy (energy of state) which they will liberate in one form or another during their breakdown" /18, p. 82/. Moreover, due to a series of physical causes, conditions are created inside UMC for the migration of energy throughout the constellation, with temporary accumulation in certain regions; in rare cases, the energy potential can rise to the level of the ultra-violet. The argument for the presence of UMC in the molecular substrate under investigation might be supported by the migration of energy to fairly remote distances (deliberately supramolecular), or by an isolation of energy when energy inflow is disconnected.

Let us examine first the results of model experiments in protein solutions. In these investigations,[17] the protein molecules were oriented either in a rapid flow or in a capillary along which a weak electric field was created, or in a strong magnetic field. Occurrences suggesting the existence of UMC were observed in all cases: in the first two cases, a so-called secondary irradiation was observed by the method of mitogenetic analysis (i. e., by the use of UV-irradiating yeast as a biological detector). This can only be the result of a migration of energy along mutually oriented molecules. In experiments with the magnetic field, energy, supplied from outside in the form of an infra-red irradiation, was added to the ultraviolet level; the ultra-violet irradiation of mitogenetic intensity increased significantly upon periodic exclusion of the orienting factor (in an intermittant field).

These simple models illustrate those dynamic conditions which are presumed to exist in the protoplasm whenever UMC are found. These, still unknown, conditions should act in a similar way in living systems in orientation and regulation of molecular movement.

The presence of UMC in living systems was proven empirically by demonstrating the effect of degradative mitogenetic irradiation /18, 19/. All the animals and plants investigated for this purpose showed an outburst of irradiation following a sudden disturbance in the normal metabolism induced by intense cold or by light anesthesia, or by mixing the intracellular structures by centrifugation, or finally, by brief exposure to a weak alternating current. If, however, two of these "degradative" influences are applied in rapid sequence, then there is no further outburst of irradiation. Some time after cessation of the influence, full physiological restoration follows, i. e., all "degradation" states are fully reversible. These data, together with others too lengthy to present in a brief survey, show clearly that the degradative irradiation is connected with the disruption of unstable (nonchemical) molecular bonds. Hence, the conclusion that UMC are present in the protoplasm.

If we recognize the existence of unstable molecular structures in the cell, then we have to agree that certain orienting factors are present. They act on the molecules and can, to a limited extent, be compared to a rapid current or either to electric or magnetic fields. In other words, it follows that the molecules of the protoplasm are in the sphere of action of a certain orienting field.

This is the empirical basis of the theory of the cellular field; its later postulates are of a more inductive nature, and we shall examine them below.

Main postulates of the theory on the cellular field

We have already formulated the first postulate: the molecules in the protoplasm are objects of action of a certain field. In other words, apart from their irregular Brownian motion, they are under the influence of a factor which is equal in size and direction to the vector of the field in the given point.

Here we arrive at the main difference between the biological field and the physical fields: it is assumed that the energy of the biological field is not concentrated in a single "source" because "local" energy resources are used in any case, i. e., part of the energy of excitation of the molecule is in the sphere of action of the field. The field transforms part of the energy of excitation of the (protein) molecule into kinetic energy. The ratio of this directed energy to the total energy of excitation of the molecule forms the degree of intensity of the field in the given point. An important conclusion, among others, derived from the above, is that the field does not exert any influence on nonexcited molecules.

In this instance, the question on the field source is not an open one. It is accepted that the field source is a molecule of chromatin (deoxyribonucleoprotein) at the time of its synthesis (formation of the complex between DNA and protein) in an existing field. Consequently, the field sources are localized in the cell nucleus; the nuclear field is continuous (somehow transmitted from one chromatine molecule to another) and is formed of separate, alternately "bursting," elementary sources. In view of the vast number of chromatin molecules, the overall field source is assumed to be in the center of the nucleus. The field source has a repulsive effect on the excited molecules; similarly, the different field sources repel one another. The size of the field vector decreases with the increase in the distance from the source. The extent of the inverse ratio cannot be determined without empirical verification, but it is most probably a hyperbolic relationship of the first or second order.

It is further assumed that each source surrounds itself with an anisotropic field, i. e., the vectors of the field are dissimilar in the three different dimensions. This can be expressed by drawing around the field source an ellipsoid with three unequal axes. The anisotropic axes of elementary ("explosive") fields are parallel within the same nucleus. The configuration of the anisotropic ellipsoid is accepted as a constant characteristic for the species and, according to the field theory, it is the only hereditary predeterminant from which the diversity of all organic forms is derived solely through epigenesis. This completes the axiomatic part of the field theory.

The unusual nature of the above postulates, combined with claims for them of biological universality, is sufficient to arouse frequent distrust and indifference. Without attempting to persuade the reader, we shall try to clarify the problem somewhat in the following way: first, a simple comment on the compatibility of the postulates of the field with the basic laws of biology and physics; second, an investigation of the essentially new possibilities opened by adaptation of the "field principle" to molecular and cellular processes in living systems.

The first problem is that of the compatibility of the "field concept" with the basic principles of physics. We do not consider ourselves competent to examine this problem in all its detail, and we shall, therefore, limit ourselves to the statement that the "field concept" does not contradict the first law of thermodynamics (the law of conservation of energy). As for an application of the second law to "open" systems, such as the biological one, we can only say that this is an unsolved problem. Gurwitsch's statement, presented here, helps to clarify the relation of the field to physical laws and gives a brief and general expression of its activity in living systems.

"A prevalent conviction," writes Gurwitsch, "is that various chains of events leading to known life-phenomena will be reduced in the remote future to the level of those parameters which operate satisfactorily in physics and chemistry.

"In opposition to this conviction we propose the hypothesis that another, higher and more complex level is operating, which is specific for life-phenomena, i. e., that biology has its own specific parameters, which, although comparable to those of physics and chemistry, are not identical with them.

"The 'field theory' is an attempt to give a formulation of these parameters... The "field concept" is not derived from physical data, but from physical possibilities. These possibilities are denoted in physical terms. In this respect, the field theory creates a new precedent, which, however, does not conflict with basic physical ideas and experiments. The construction of physical possibilities in physical terms underlies all constructions of successive events leading to phenologically independent processes of embryogenesis, and to repeated processes (periodical and aperiodical) which occur continuously throughout the life cycle. Even if the 'field theory' is considered as a basic 'parameter' specific for all living systems of 'the given', it still would not follow that all processes occurring in living systems are identically determined by or dependent upon the field. Such a primitive idea is nothing more than sheer tautology. On the other hand, the formulation of the field is well defined and delimited. The field has . . . the meaning of a regulating invariant of steric parameters which describe molecular processes taking place in living systems" /7. pp. 249-251/.

We shall now say a few words about the biological plausibility of the main postulates. The use of local energy by the vector field substantiates the fact that the energy resources of the cell are not in any case concentrated wholly in the nucleus. Connection of the field with the molecules of chromatin is an expression of the finally-proven hereditary role of the nucleus in the "field theory," although the mechanism of action of the nucleus on the protoplasm is, of course, understood quite differently than in classical genetics. In translating the expression "chromatin molecule" from the language of cytology to that of biochemistry, Gurwitsch considered that it is most plausible to relate the "outburst" of the field with the formation of the DNA-protein complex (in an existing field). This principal statement of the continuity of the field (its transmission from one molecule of nucleoprotein to another) essentially postulates that self-inheritance is not conceivable from the point of view of the "field theory." A consequence of the main postulates, which is often forgotten, is that the intensity of the overall nuclear field does not depend upon the total amount of chromatin in it, but on the intensity of its turnover.

We shall now examine the rational aspects of the field activity on the molecular and cellular levels. The efficiency of the field is always demonstrated by the conclusion which follows naturally from these general postulates: (1) the sphere of action of the vector is not limited by the boundaries of the cell, which contains the source; and (2) generally speaking, it is not strictly related to the size of the given cell. Therefore, it diminishes in all directions in proportion merely to the distance from the source and depending upon the orientation and anisotropy of the source. This makes it possible to speak about synthesis of the fields of two or several neighboring cells; in every point (both intra- and extracellular) the resultant vector is expressed by the geometrical sum of the vectors of all nuclei obtained by the rule of the parallelogram. The synthesized field of the cellular complex is called by Gurwitsch the "actual field."

Analysis of actual fields

The molecular and submicroscopic levels. In an isolated cell the field source has a repellent effect on the excited molecules of the protoplasm; at the same time, the unexcited molecules concentrate in the prenuclear region. This relationship between the nucleus and the protoplasm molecules indicates that the most balanced state of the nucleus is achieved when it is in the geometrical center of the cell (with similar concentrations of excited molecules in each direction). Any beveled configuration of the cell will be unbalanced and will strive toward a more symmetrical configuration (Figure 11).

If, at least, two field sources are interacting (both the nuclei of various cells and various chromosomes of the nucleus and the centrosome[18] in the same cell) it may lead to important consequences: the geometrical configuration of opposite vectors will create a zone where the field vector is zero. This zone will appear at a specific distance between the sources which will be closer to the weaker source (Figure 12). The excited molecules are directed to this zone by the intersecting vectors and stay there as long as they do not lose their energy of excitation and do not leave the sphere of action of the field. This is the simplest example of a stabilizing field creating a so-called zone of molecular concentration.

In Gurwitsch's conceptualization, these zones play an important physiological role. Unstable molecular constellations (UMC) formed in these zones accumulate the high potential energy of several adjacent molecules; they can be defined as dynamic accumulators of cellular energy. Equilibrated chemical complexes are formed, when several reactive molecules approach one another. In certain cases, these complexes may

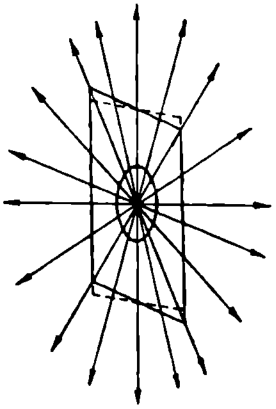


FIGURE 11. The field of a beveled cell

The curve passing through the distal ends of the vectors affixed to various points on the surface of the cell forms an ellipse, the axes of which correspond to the axes of anisotropia. The lateral walls of the beveled cell are symmetrical in relation to the field; the base and top are asymmetrical, and therefore the field has a tendency toward "straightening," as illustrated by the dotted line /6/.

"grow up" from the purely chemical into the submicroscopic and microscopic spheres (the so-called vestigia or irreversible "traces" of field action). Thus, molecular concentration in regions of accumulation are mostly avestigial and lead only to the formation of fully reversible UMC, although certain vestigial processes may lead in final analysis to the formation of large cellular structures or organoids. It is important to take notice of this natural sequence, in which, according to Gurwitsch, the chemical processes become optically visible ones.

On a suitable substrate, UMC and vestigia can also appear beyond the limits of the cell in the neighborhood of sufficiently strong stabilizing fields. This explains the extra-cellular formation of a series of fibrillar structures.

Fairly long peptide chains which lack mobility become deformed rather than displaced under the effect of an actual field with several sources. The nature and degree of the deformation depend on the mutual disposition of the field sources.

Interaction between various sources of the field on the cellular level. Merely on the basis of indirect data, one can discuss the action of the field on the molecular level. (The effect of "field factors" at higher levels is obvious.) Most clear-cut data were obtained from an analysis of the interaction between chromatin figures of neighboring cells in several meristems /21/. The chromatin figure is a powerful field source, especially at the stage of spireme, when the resultant of the field vectors passes through the axis of symmetry of the vectors. If the chromosome figure of a neighboring mitosis happens to be somewhere along this axis, one would expect it to turn under the action of the repellent field of the spireme as a result of which the axis of the given mitosis would be displaced This, in fact, does occur (Figure 13) with a large quantity of material.[19]

All details of chromosome behavior during mitosis can be derived from the "field hypothesis"; the theory of mitosis is discussed in a large amount of unpublished data of Gurwitsch, now in his personal archives.

Finally, the previously investigated deformation of nuclei in the cartilage primordium (see p. 65) can also be considered a result of the repellent action of the nuclei themselves as sources of the field. The configuration of the actual field in the cartilage rudiment (the center of which can be reduced to the center of the rudiment due to a radial symmetry) shows a similar picture to that obtained from Anikin's calculations based on premises of some completely abstract invariant /6, pp. 71-72/.

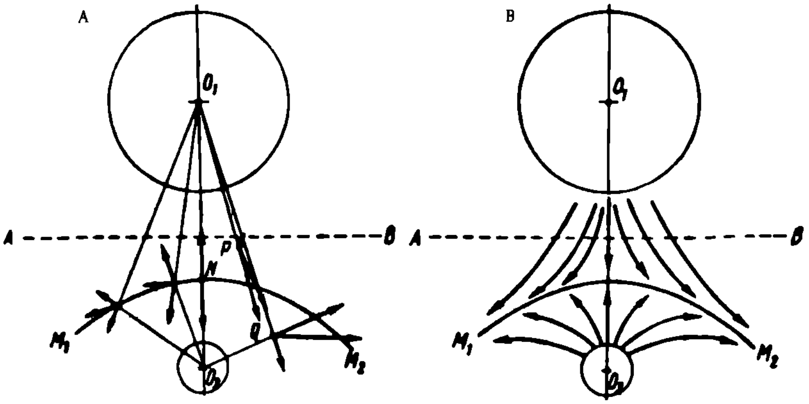


FIGURE 12. Formation of the "zero zone"

A — diagram of vectors for several points; B — general picture: O_1 and O_2 — sources of isotropic fields, the intensities of which are proportional to the diameter of the circumscribed circles; AB — average line which may pass through the boundary of the cells with the nuclei O_1 and O_2; M_1M_2 — zero zone; in point N, the resultant of the vectors equals zero; in other points of the zero zone the resultant is directed along the tangent, while in points P and Q outside the zero zone, the resultants are directed to the side of the latter /7/.

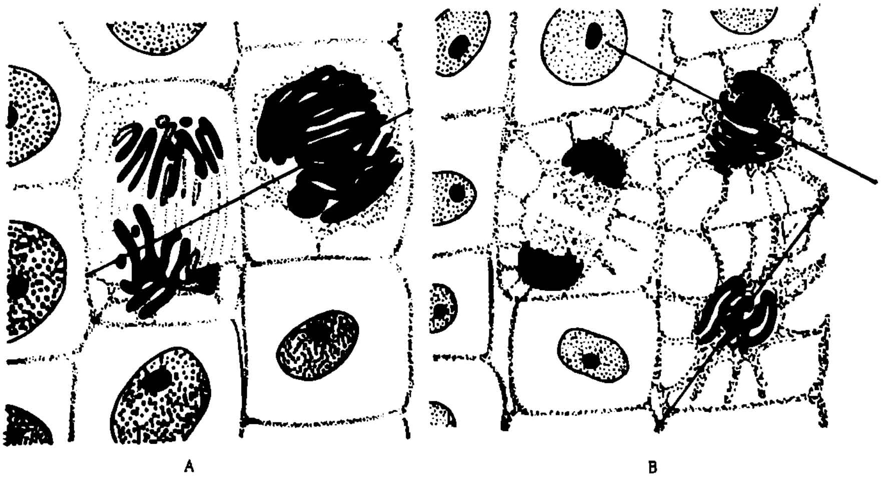


FIGURE 13. Results of the action of the spireme on the neighboring ana- and telophases

A — displacement of the mitotic axis which continues the spireme axis; B — symmetrical telophase not located in the continuation of the axes of neighboring spiremes /21/.

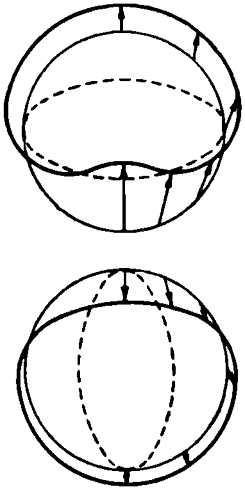


FIGURE 14. Diagram of the interaction of cellular fields from two isolated cells

It is assumed that the cells have a spherical form. The anisotropy of their fields is indicated by the ellipse circumscribed in the cell; the large axes of anisotropy are perpendicular to each other /6/.

Action of actual fields on the cellular level. We shall present here only certain results of a detailed analysis by Gurwitsch, most of which is unpublished /7/ although some of it appears in "Theory of the Biological Field" /6/.

The displacements or deformation of whole cells under the influence of field vectors should be regarded as a result of displacement of the intercomposition of cells. Some purely mechanical properties (the incompressibility of the cell) play an important role here.

Analysis of the simplest case of actual field, the interaction between two cells (nuclei), shows that the latter separate and, at the same time, undergo deformation under the effect of interacting fields of the cell (nucleus) (Figure 14). Cessation of the mutual repulsion of cells is either a consequence of the weakening of vectors with an increase in distance, or, which is more frequent, of the counteraction of strong external fields (for example, of the epithelial rudiments). Analysis of the action of epithelial fields and of fields in large cellular complexes is of great interest (see below).

It can be easily shown that the effect of the field of an epithelial layer on some outside point depends largely on the extension and, in particular, on the configuration of the layer (Figure 15). The field effect of an extended layer at a distance equal to the width of the layer is nearly constant; so that it can be referred to as the distant action of the field (Figure 16).

The action of the field of a plane (two-dimensional) cellular accumulation on some point inside it differs from the action of the field of a three-dimensional cellular accumulation in the following important respect: in the planar accumulation, almost all cells, except for the most peripheral ones, are under similar conditions of the field. The latter are, in fact, not a function of the cell coordinates in relation to the center of the layer; in the three-dimensional (spherical) accumulation, the field vector on a certain internal point changes markedly with the change in the coordinates of the point, as seen in Figure 17.

The bending of the ectoblast under the effect of its own actual field has a clear-cut interpretation. One can see in Figure 18A, that an insignificant, initial shift in one of the cell nuclei, in relation to the level of disposition of its neighboring nuclei, suffices for the formation of a continuously growing convexity, into which the neighboring cells are gradually attracted one after another (Figure 18B). The following factors limit the autonomous growth of the convexity: a) appearance of an external opposite field, for example, the approach of ectoblast; b) growth of the apical "repellent" vector to such an extent that the cells at the top of the convexity get "cut off" and the convexity as a whole stops growing; c) some, more specific factors, which are not directly connected with data of the actual

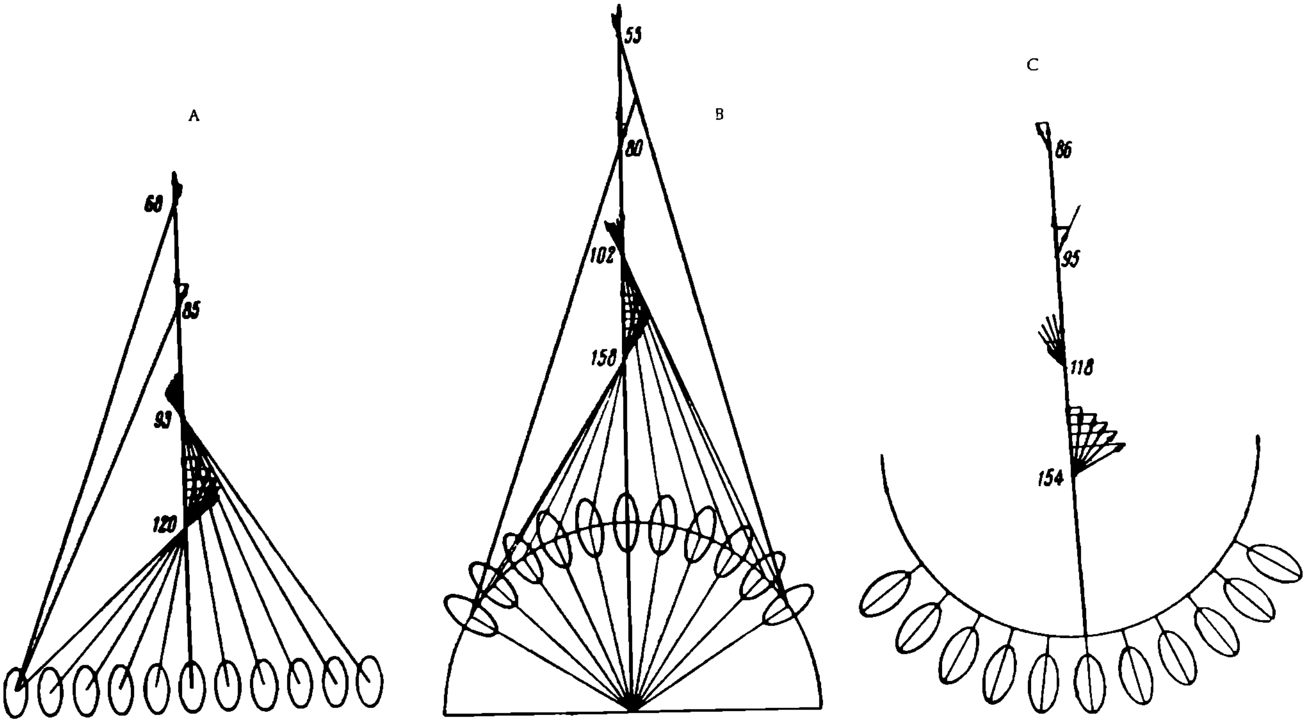


FIGURE 15. Actual field formed in layers of various configurations

A, B, C — the field vectors in four equidistant points from straight, convex, and concave layers /6/.

field, (e. g., the amount of the mobile cellular material). Analysis of a few actual cases (various types of gastrulation and of displacement of brain vesicles) shows that the species specific anisotropy of elementary fields can play an important role in the species differences noted in the methods of invagination. Change in the strength of the intecellular links can also, of course, change the external picture of the morphogenic process which takes place when there is a similar distribution of field effects.

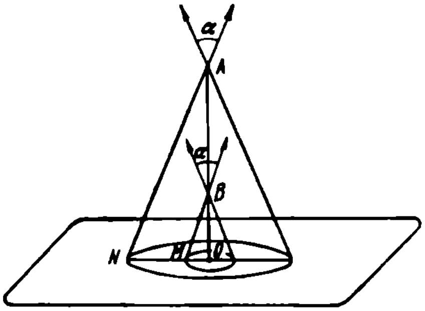


FIGURE 16. Diagram showing the virtual nondecrement of the field on a layer of infinite extension

If field vectors, which do not deviate too markedly from the normal to the layer, are situated inside angle then the distance from the layer through the passage B to A is compensated by the incipient action of vectors of cells of the MN ring /7/.

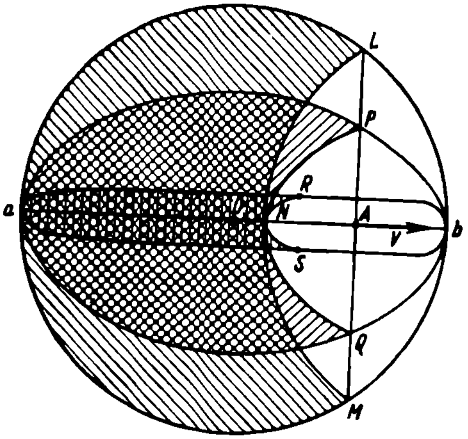


FIGURE 17. Change of action of the actual field of a cellular complex in point A following the compression of cells

If the complex has a spherical shape with the surface LaMb, then the vector v directed to the right of the cells situated in the volume LaMN acts on point A. (The action of volumes ALNM and ALbM, which are equal to A and equidistant from A, are mutually cancelled.) If the complex has the shape of the ellipsoid bPaQ, then, by virtue of similar considerations, the vector on point A issues from volume PaQn; in the case of the planar complex aRbS, it is the volume RaSN which is active. Since $V_{RaSN} < V_{PaQN} < V_{LaMN}$, and the center of gravity of the complex acting at the left moves farther from A during compression of the latter, the value of the vector v decreases. This means that, following compression of the complex, a point which maintains the same position relative to the center of the complex "feels" its asymmetry less /7/.

Another conclusion follows from the analysis of actual fields: in certain special cases of interaction between two neighboring sources of very different size, their mutual repulsion can be compensated, or even heightened, by "recoil" from the surrounding, excited molecules of the protoplasm, and then the sources will draw closer.

The following is only one of the numerous examples of morphogenesis analyzed by Gurwitsch: differentiation of the brain from the primary smooth wall of the neural tube. Figure 19 shows that the localization of the bulgings can be accurately predicted from a precise drawing of the preceding stage. The slightly delineated bendings will grow deeper. From the structure of the given actual field, one can easily derive the predicted characteristic nuclear rotations and displacements. This obviates the need for a dynamically preformed morph which, at present, should be considered merely as a preliminary statement.

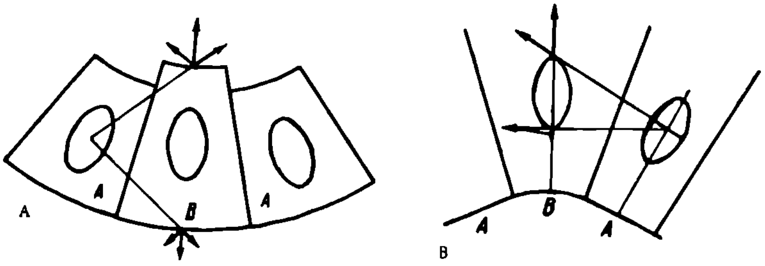


FIGURE 18. Appearance and growth of an inflection in the ectoblast under the effect of its own field

A — effect of the field of cells A on larger cell B, the nucleus of which is situated farther from the base of the cell. The vector directed to the concave side predominates. (In the construction, the anisotropy of the fields of each nucleus has been considered; it is expressed by means of ellipsoids which delimit the nucleus.) B — effect of the field of cells A on cell B, following a certain amount of invagination /6/.

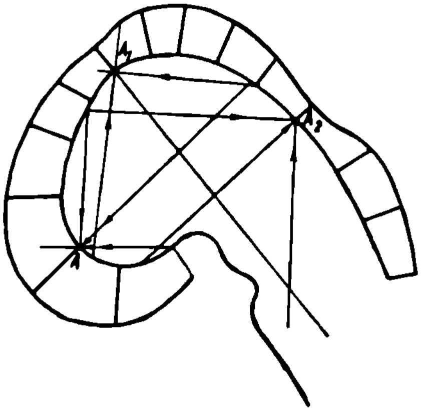


FIGURE 19. Simplified diagram of the field of brain vesicles

The inside contour represents a medial section through the brain of a two-day old chick embryo. The interaction between different parts is arbitrarily shown by means of three vectors: one normal to the examined point, and two forming an angle of 45° with the first. Consequently, the primary brain vesicle is divided into the anterior, middle and posterior brain /6/.

This is a good example of the unexpected but workable potentialities of predicting the results of a given morphogenesis, made possible only by applying the "concept of the actual fields," and based on general postulates (which also have a broader application).

To understand the relationships of the field pattern to "induction," "determinant" and other agents of the mechanics of development, let us examine a case which, at first glance, presents unsolvable difficulties to the "field concept": the chemical induction of the nervous system.

Experimental embryology has toiled to explain the appearance of a natural form resulting from a purely chemical stimulus. This question is either overlooked or postponed, and it is here that the "field theory" can be helpful. In order to demonstrate the possibilities of the "field principle," let us start with the assumption that penetration of the inducer molecules into one or several cells of the "competent" outer layer causes their swelling and displacement from the row of neighboring cells. The resulting pattern and further development of the process is similar to that shown in Figure 18. The parameters of the actual field are in continuous evolution, which ensures the irrepressibility of the process, while the spatial specificity of the result derives from its constant regulating factors (anisotropy).

This example shows that, on the basis of the "field principles," specific results of morphogenesis can be derived from small (possibly, purely chemical) initial changes. These are sufficient to cause a slight initial asymmetry in the actual field, and the process goes on automatically.

Concepts of the whole, of rate-setting and of the invariant in the theory of cellular fields

It may seem that the "theory of cellular fields" completely denies the principles of integrity of morphogenesis, of the presence of certain degrees of freedom in the behavior of elements, and of the rate-setting invariant laws. It may even seem that actual fields are a regression to an almost Virchowian segregative-deterministic point of view. However, careful examination /11/ shows that this is not so, that the "concept of actual fields" is merely a marginal definition, and an approximation of the requirements for investigating the principles of action of "the whole" on the elements.

In any construction of an actual field, the vector acting on any cell is not the arithmetic sum of the vectors of separate cells, but their geometric sum: it depends, first of all, on the geometry of a given complex, i. e., on a property related to the complex just as it is to the whole. This can be seen in any of the examples above, and is, in fact, the formulation of the "principle of the whole" as it appears from the viewpoint of the "actual field." Obviously, the field vector has merely a rate-setting effect on the molecules of the protoplasm and, through them, on the cells, since, according to the principal "law of the field," only part of the total energy of excitation of the molecules is transformed into directed energy.

The next problem is one of translating the invariant principle into the language of the cellular fields. The invariant is no longer seen as an abstract geometric law of unknown origin, relevant solely to the embryo, but a universal principle of regulation of life processes, which is formed of the following components: a species constant (anisotropy of the field) and variable parameters (the state and intensity of various sources). In the new concept, the action of the invariant is not limited to some arbitrary period of development. ". . . in any phase of the system a discrepancy occurs between some of its variable parameters and the invariant, so that the process tends to decrease or abolish the discrepancy" /6, p. 178/. If the constant (anisotropy) is Π, the variable parameters (intensity and disposition of the field sources) are x, y, the field vestigia in the most general sense (i. e., all features of a real configuration) are v_1 and v_2, and the invariant is I—, then a very general expression can be suggested for the interaction of the field with the substrate:

$$\begin{tikzcd}
I = (\Pi, x, y) \arrow[rr] & & v_1 \arrow[dl] \\
& v_2 \arrow[ul] &
\end{tikzcd}$$

This means that the invariant acts on the v_1 vestigia, transforming them into the state v_2; this leads automatically to a shift of the variable parameters of the actual field, which act again on the present vestigia, and so on. This expression is called by Gurwitsch the "principle of conjugation," and in various forms it can be applied to both irreversible and reversible physiological processes. Unfortunately, we cannot present the contents of these structures in this abbreviated form.

CONCLUSION

We shall now present a few conclusions derived from this survey of the history of the biological field concept. We shall try to answer the following questions: 1. what is the relation of the "field theory" to experimental data, and to the general trend of modern biology (mainly, embryology), and 2. what is the place of the "field theory" in causal biology.

In answering these questions, it should be kept in mind that the "concept of the field" is a special one. It has developed along lines other than those of experimental embryology. Since the "field concept" was designed to fill gaps overlooked by experimental embryology, it is clear that the "field principles" can neither be confirmed nor disproved by modern experimental methods. These only establish the presence or absence of a connection between the most variegated initial influences and the form of the embryo, without dealing with the problem of the creation of this form out of deliberately nonvectorial influences. Only some auxiliary aspects of experimental investigations are of interest to the "field theory" as for example, the construction of cellular associations. In particular, it has been firmly established that a concentration, or contrariwise, a dispersal of cells plays an important role in the differentiation of explants. It would be interesting to analyze this from the point of view of the actual fields of differentiating complexes.

The recent proliferation of cinematographic studies are, however, of much greater value in the development of the "field theory." The intermediate stages of formation (including lability and randomness) which lead to an accurate morphological result can only be observed with cinematography. The work on the cleavage of ascarids [20] performed by a group of French authors points to the high lability of the protoplasm and the mitotic figure, which result in an accurate form only after numerous "inaccurate" attempts. The works of Gustafson and co-authors describe in detail similar processes during gastrulation and the subsequent development of the sea-urchin.[21] Certain conclusions of Gustafson are of particular interest to us. In the section entitled "Problems of coordination and accuracy," he writes "One of the most fascinating peculiarities in the development of the sea-urchin as a whole is that, unlike the final result, which is relatively accurate and constant, the processes leading to it show marked variability. For example, the final architecture of the skeleton of Pluteus, although showing some variations, is considerably less variable than the movements of the separate mesenchymal cells forming it. . . A relatively constant final result can be achieved in a multitude of ways. . . This observation suggests that accuracy can be determined, not by a single process, but, on the contrary, as the result of several processes, each on a low level of accuracy, which, owing to mutual compensation, accords a certain accuracy to the process taking place in a given region."[22]

It is superfluous to emphasize how close such statements are to the principle of "rate-setting" of the elementary processes.

A survey of the developmental course of purely experimental embryology over the last 20–30 years, seen from the point of view of "the field," brings one to an interesting conclusion, insufficiently realized by most investigations: that is the growth of evidence of the autonomous, yet reciprocal, character of the components of morphogenesis. The great importance of this conclusion was visualized by Spemann, who spoke about the "synergetic principle,"[23] by Filatov, who used the term, "complexity"[24] and by others who preceded them. The main point of these authors is that indirect "inductive" influences of one part on another cannot be regarded as sole and sufficient "pivotal" factors of development. On the one hand, they cannot act successfully without a certain readiness of the reacting material (labile determination) which appears independently of any inductor. On the other hand, in the absence of a specific inductor, the reacting primordium often develops in the same direction, although, not exactly to the same extent as when in contact with the inductor. All this indicates that there exists some kind of preliminary interrelationship or symbasis (synergism, according to Spemann) in the behavior of both parts of the primordium; it is impossible to explain this interrelationship by a strict one-sided action of one-part on the other. Recent investigation showed that similar occurrences are not isolated cases. A quantity of data has accumulated on the separation of those processes which normally take place simultaneously, and which had previously been thought to be absolutely and closely bound. Some of these data are mentioned and analyzed in an interesting review by Aleksandrov.[25] It became clear that no direct relationships, similar to induction, or to the relationships between the components of a chemical reaction, exist between the elementary processes which make up even such a highly-coordinated act as mitosis. But then, the problem of the factors of coordination of these processes, in time and space, acquires a new aspect: it becomes necessary to find the factors which connect these mutually independent processes, or which, at least, possess a certain mutual degree of freedom.[26]

An unprejudiced approach to modern experimental morphology is required to make the necessary search for the determining factors of mutually interrelated elementary processes. This is the approach of non-determinism, of rate-setting of the elementary processes, which leads to a search for a regulating invariant acting "at a higher level." In our view, the wide distribution of this type of coordinating mechanism, new to biology, should be taken as a serious argument in favor of the "field principle."

Although the authors' observations might well have brought them closer to the "field principles", in fact, they did not. Contemporary investigators can be arbitrarily divided into two groups according to their perspectives of theory. One group is oriented around a field theory and is engaged in vague searching for "supra-cellular growth centers,"[27] in attempts at elaboration of concepts of cellular tropisms or taxis[28] and in attempts to distinguish "stimulating" and "controlling" factors of development.[29] The more general tendency, however, is to mix the principles of the sought-for theory with certain empirical facts, as Child has done. There are numerous supporters of the views first presented by Holtfreter on the decisive role of selective cellular affinity in morphogenesis. It is doubtful whether selective affinity between cells which have already undergone differentiation can be a suitable background for a general theory on morphogenesis. It is clear that any attempt to derive a theory from this, if only to explain the elementary processes of invagination-evagination, would degenerate into sheer tautology. To use the expression of Gustafson[30] the author of these views, "the geography of the forces of selective affinity" in the embryo cannot be derived from any fractional analysis of these forces. It is interesting that Holtfreter regards the successful outcome of neurulation to be dependent upon a "happy coincidence" in time between certain well-determined motor tendencies of cells and the appearance of certain forces of contact affinity.[31] It would seem that a 100% repetition of this "happy coincidence" in normal embryogenesis would be enough justification for seeking a theoretical explanation. It is impossible to formulate an explanation of this event, however, in terms of forces of selective affinity, because the entire phenomenon is only another example of synergism of experimentally distinct processes. Undoubtedly, a thorough analysis of cellular contacts, as performed by Weiss,[32] for example, could supply the field theory with reliable experimental data.[33]

In some respects the situation is similar, in others, dissimilar, in the field of molecular biology. A physiological theory of the protoplasm is widely accepted without question, although its authors are often forgotten. However, most scientists in the fields of biochemistry and cytochemistry continue to ignore the physiological aspect of molecular processes taking place in the protoplasm, and content themselves with representations of balanced, stable structures. The need to look for certain general orienting factors acting on the molecular level is apparently barely recognized.

Thus, although the conclusions derived from the "field theory" are in agreement with experimental data of modern biology, and experimental evidence supports the "field principles," theorists do not yet accept the "field idea". It is unlikely that pure experimentation would produce "field principles", because biological experiments can only reveal initiating factors in a process, but not its specific result. The history of the "field theory" is a long search for the most concise and most widely accepted descriptive statements on the prime factors of the process. From these, then, specific results can be derived. Stated in general terms, the prospect of the "field theory" is the creation of structures for invariant leading factors applicable to the most variegated biological processes which take place on the cellular or molecular levels, or on both levels at the same time. If the right structures are found, this will enable us to predict, from a few initial conditions, the course of morphogenetic processes in a developing embryo. In certain cases this is already being done by means of actual fields. When the "field idea" is applied to reversible physiological processes and purely molecular processes (where the possibilities of prediction are less obvious), the first result will be advanced knowledge of unequilibrated molecular structures in the protoplasm and, as a consequence, advanced knowledge of the nature of evolution and transformations of purely vestigial (balanced) structures.

The future of the "field theory" depends upon recognizing the possibility and importance of finding leading invariant factors for every biological process. These factors are related to common initial factors (now under study) in the same way as the formula of a body's movement is related to the source of the force which causes the movement. It should be understood that the whole complex of events cannot be separated from the model of the "start;" that the leading factor cannot be replaced by any summation of all possible "start" factors; and that the field vector is not merely one of several agents which act "apart from" inductors, gradients, genes, and so on, but is a formulation of the only conditions from which the specific result of the initial actions can be derived.

The "field theory", then, was developed using embryological material, and is an attempt to extrapolate the "embryological way of thinking" to biology as a whole. However, it is the "biochemical" way of thinking which prevails in contemporary biology. This way of thinking is not accustomed to the requirement for invariant principal conditions, but is trained, instead, to the "chain" method of deriving a certain step from the one preceding it, and extrapolating this method to other fields of biology as well. It is possible, however, that further development of biochemistry and of other fields in molecular biology will lead to a recognition of the inadequacy of this method in explaining the coordination of molecular processes. In other more "embryological" fields, the gap between our ability to isolate certain specific results and the variety of possible meanings of relationships between initial factors and final results has grown to such an extent that it is now being recognized. The theoretical views of Gurwitsch will find their place in the biology of the future when it will be recognized that such "nonclassical" statements as the "field postulates," far from being dim or contradictory, should be utilized in order to find simple laws that will fill these gaps.

Notes

[1] An exception to this rule is apparent in the cleavage, the determinism of which does not fully disappear up to the stage of gastrula.

[2] Goldschmidt, R. Physiologische Theorie der Vererbung. Berlin. 1927.

[3] Waddington, K. Organizatory i geny (Organizers and Genes). [Russian translation. 1947.]

[4] Sinnot, the great American botanist, comments on this problem in his study of the action of his "genes of the shape of the fruit" in squash: "Most works on the action of genes report that the gene controls the secretion of specific substances: the question arises how does the substance determine the development of a specific shape? One may think that the substances secreted by the gene act like inductors, stimulating or modifying the formative possibilities of the living matter in the organism. We have to accept the presence in the protoplasm of something inherent to it which, in the absence of something better, can be compared to a three-dimensional system. It can be speculated that

the specific substance somehow deflects or modifies the coordinates of this system. The problem consists of perceiving this fundamental formativeness. Calling it a field does not yet explain it . . . But even if it cannot be explained, the mere recognition of its existence is a step forward, and this can save us from going too far in the naive speculations about the nature of action of genes in the course of development" (Sinnot, E.W. Plant Morphogenesis, p.425. New York, 1960).

[5] This viewpoint, expressed 45 years ago, would today be called cybernetics.

[6] Activité et travaux du laboratoire d'Embryologie Expérimentale du Collège de France, No. 8. 1958–1959.

[7] Grobstein, C. Differentiation of Vertebrate Cells. — In: The Cell. New York-London. 1959.

[8] Harrison, R. Nekotorye trudnosti problemy determinatsii (Certain Difficulties in the Problem of Determination). [Russian translation. 1934.]

[9] This alternative is usually complicated by the interaction between elements which is shown by accurate analysis to be dependent upon the other two mentioned above, i. e., the results of interaction between elements can be determined either by previously determined parameters, which leads to statement (I), or to their situation in the field, which leads to statement (II). In modern embryology there are few attempts at a logical "deduction" from the characteristics of the interacting elements to the final results of morphogenesis, but wherever this is done the interaction is always conceived from a preformistic viewpoint. Interaction viewed from the standpoint of the field principle forms the content of the second version of the field theory ("actual fields") /11/. In the initial version, which is examined in this chapter, the direct interaction between elements is replaced by their subordination to a common field.

[10] Such criteria of the behavior of elements as mutual orientation, shape, density of elements, etc., are capable of supplying direct information on the nature of action of factors of "the whole" on the elements. The a posteriori experimental proof of the equipotentiality of elements, which is commonly used in the mechanics of development, is not applicable in this case.

[11] The cybernetic "rule of diminishing diversity" is quite similar to the above (notes 3 and 4). If a certain set is repeatedly subjected to a single-valved invariant transformation, the diversity of the set decreases; the originally diversified sets are reduced to a less differentiated finality. Thus, in given systems the equifinality of their behavior is a sufficient criterion for their prolonged presence in the field of action of invariant factors.

[12] Polezhaev, L.V. Osnovy mekhaniki razvitiya pozvonochnykh (Principles of the Mechanics of Development of Vertebrates). — Izdatel'stvo Akademii Nauk SSSR, pp.253–257. 1945.

[13] Spemann, H. Experimentelle Beiträge zu einer Theorie der Entwicklung. Berlin. 1936.

[14] Gurwitsch, A. Über Prämissen and anstossgebende Faktoren der Forschung und Zellvermehrung. — Arch. für Zellforschung, Bd. 2, S. 495–548. 1909.

[15] Dorfman, V. A. Khimicheskaya promorfologiya yaitsa (Chemical Promorphology of the Egg). — Uspekhi Sovremennoi Biologii, Vol. 43, pp. 55–69. 1957.

[16] A ball which is maintained in the air by means of a fountain stream serves as a simple mechanical model for an "unstable constellation."

[17] Gurwitsch, A. G. Popytka sozdaniya modeli neravnovesnykh molekulyarnykh konstellyatsii i degradatsionnogo izlucheniya (Attempt at Creating a Model of Unstable Molecular Constellations and of Degradative Irradiation). — In: Sbornik rabot po mitogenezu i teorii biologicheskogo polya," pp. 92–101. 1947.

[18] Gurwitsch refers to the opinion of several cytologists who consider the centrosome a derivative of the nucleus; it would follow from their viewpoint that the centrosome is a field source, although a much less intensive one than the nuclear one.

[19] For example, out of 339 single mitoses, only 35 were asymmetrical; out of 115 mitoses in the neighborhood of spiremes and in the continuation of their axes, 113 were asymmetrical; out of 303 in the neighborhood of spiremes, but not in the continuation of their axes, two were asymmetrical, and the rest symmetrical (data from the root tip of onion).

[20] Nigon, V., P. Guerrier, and H. Monin. L'architecture polaire de l'oeuf et les mouvements des constituents cellulaires chez quelques Nematodes. — Bulletin Biologique de France et de Belgique, t. 93, pp. 132–202. 1960.

[21] Gustafson, T. et al. Studies on the Cellular Basis of Morphogenesis of the Sea Urchin Embryo. — Experimental Cell Res., Vol. 21, pp. 361–373. 1960; Vol. 22, pp. 437–449. 1961; Vol. 24, pp. 64–79. 1961; Vol. 25, pp. 311–325. 1961.

[22] Ibid, Vol. 25, p. 323. 1961.

[23] Spemann, H. Experimentelle Beiträge zur einer Theorie der Entwicklung, S. 59.

[24] Filatov, D. P. Sravnitel'no-morfologicheskoe napravlenie v mekhanike razvitiya, ego ob"ekt, tseli i puti (The Comparative-Morphological Trend in the Mechanics of Development, Its Object, Aims and Methods). — Izdatel'stvo Akademii Nauk SSSR. 1939.

[25] Aleksandrov, V. Ya. Problema avtoregulyatsii v tsitologii. Organizatsiya mitoticheskogo deleniya kletki (The Problem of Self-Regulation in Cytology. Organization of the Mitotic Cleavage of the Cell). — In: "Tsitologiya," Vol. 4, pp. 3–15. 1962.

[26] It is surprising that, while mentioning Wilson and Bovary among his predecessors, the author does not include Gurwitsch, who for many years developed and propagated his views on mitosis as a comprehensive organized "structural process."

[27] Berrill, N.J. Growth and Form in Gymnoblastic Hydrois. VI. — J. Morphol., Vol. 92, pp. 241—302. 1953.

[28] Stefanelli, A. I movimenti cellulari nella morfogenesi. — Boll. Zool., Vol. 24, pp. 427-449. 1957.

[29] Bonner, J.T. Morphogenesis. — Princeton Univ. Press. 1952.

[30] Gustafson, T. The Forces that Shape the Embryo. — In: Discovery. Nov., 1961.

[31] Townes, P.L. and I. Holtfreter. Directed Movements and Selective Adhesion of Embryonic Amphibian Cells. — J. Experimental Zool., Vol. 128, pp. 52-120. 1955.

[32] Weiss, P. Vzaimodeistviya mezhdu kletkami (Mutual Relationships Between Cells). — In: Sbornik "Sovremennye problemy biofiziki," Vol. 2, pp. 176-184. — Inostrannaya Literatura. 1961.

[33] It would seem at first glance that the analysis of cellular relationships in vitro is critical for estimating the plausibility of the theory of cellular fields. However, this is not so: a truly repellent action can be exterted only by the sum of sufficiently strong actual fields of the entire primordia (epithelial layer, mesenchymal thickenings) where the axes of anisotropies of elementary fields are oriented in a single plane, common to the entire complex. The cellular aggregates in vitro are friable and their cellular axes are situated at random; therefore, practically, they cannot form an actual field. Naturally, the physicochemical factors of contact interaction which are not directly related to a "field," are of primary importance. Only when a dissociated cellular mass aggregates into a more or less organized whole (myxomycete, sponges, etc.), can one expect an increase in the efficiency of its actual field. The action of the latter is greater as the form acquired by this initially undetermined complex is more specific. It is, therefore, of the greatest interest to analyze cellular behavior where strong actual fields are expected.

List of works on the theory of the biological field

1. Gurwitsch, A. Die Vererbung als Verwirklichungsvorgang. — Biol. Zbl., Bd. 32, S. 458-486. 1912.
2. Gurwitsch, A. Der Vererbungsmechanismus der Form. — W. Roux' Arch., Bd. 39, S. 516—577. 1914.
3. Gurwitsch, A. Über den Begriff des embryonalen Feldes. — W. Roux' Arch., Bd. 51, S. 383-415. 1922.
4. Gurwitsch, A. Versuch einer synthetischen Biologie. — Schaxels Abh. z. theor. Biol., Bd. 17. 1923.
5. Gurwitsch, A. Weiterbildung und Verallgemeinerung des Feldbegriffes. — W. Roux' Arch., Bd. 112, S. 433-454. 1927.
6. Gurwitsch, A.G. Teoriya biologicheskogo polya (Theory of the Biological Field). — Moskva, Izdatel'stvo Sovetskaya Nauka. 1944.
7. Gurwitsch, A.G. Analiticheskaya biologiya (Analytical Biology). Manuscript from the personal archives of Prof. Gurwitsch.

8. Gurwitsch, A. Die histologischen Grundlagen der Biologie. Jena. 1930.
9. Gurwitsch, A. Über Determination Normierung und Zufall in der Ontogenese. — W. Roux' Arch., Bd. 30, S. 133–193. 1910.
10. Gurwitsch, A. Problemy nasledstvennosti (Heredity Problems). — Priroda, Vol. 8, pp. 843–862. 1914.
11. Gurwitsch, A. G. Ponyatie 'tselogo' v svete teorii kletochnogo polya (Conception of the "Whole" in the Light of the Theory of the Cellular Field). — In: Sbornik rabot po mitogenezu i teorii biologicheskogo polya. Izdatel'stvo Akademii Nauk SSSR. 1947.
12. Felicine-Gurwitsch, L. Die Verwertung des Feldbegriffes zur Analyse embryonaler Differenzierungsvorgänge. — W. Roux' Arch., Bd. 101, S. 40–52. 1924.
13. Anikin, A. W. Das morphogene Feld der Knorpelbildung. — W. Roux' Arch., Bd. 114, S. 549–577. 1929.
14. Anikin, A. W. Morfologicheskoe obosnovanie induktsii khrustalika glaznoi chashei (Morphological Substantiation of the Crystalline Induction by the Eyeball). — Trudy VI Vsesoyuznogo S'ezda Anatomov, Gistologov i Embriologov v Kieve, pp. 511–513. 1958.
15. Smirnow, E. and A. Zhelochovtsev. Das Gesetz der Alterveränderungen der Blattformbei Tropeolum majus L. — Planta, Bd. 15, S. 299–354. 1931.
16. Smirnov, E. S. Regulyatsiya formy sotsvetiya Coriandrum satirum (K voprosu o teorii polya) (Regulation of Coriandrum satirum Inflorescence Form (The Problem of the Field Theory)). — Uchenye Zapiski Moskovskogo Gosudarstvennogo Universiteta, Vol. 13, pp. 85–118. 1937.
17. Abel, P. and D. von Denffer. Über den Zusammenhang zwischen Zelle und Organpolarität bei der Ausbildung des Zellteilungsmusters auf der Blaseninnenwand von Utricularia vulgaris. — Beiträge zur Biologie der Pflanzen, Bd. 37, S. 77–84. 1962.
18. Gurwitsch, A. and L. Vvedenie v uchenie o mitogeneze (Introduction to Mitogenetic Study). — Medgiz. 1948.
19. Gurwitsch, A. and L. Mitogeneticheskoe izluchenie (Mitogenetic Radiation). — Medgiz. 1945.
20. Blyakher, L. Ya. Mitozy v yaitsakh tritona, razvivayushchikhsya posle narusheniya ooplazmy (Opyt analiza mitosa v svete teorii polya) (Mitoses in Eggs of Triton Developing after Ooplasm Differentiation) (Mitosis Analysis in the Light of the Field Theory). — In: Sbornik rabot po mitogenezu i teorii biologicheskogo polya. Moskva, Izdatel'stvo Akademii Nauk SSSR. 1947.
21. Pukhal'skaya, E. Ch. Morfologicheskie izmeneniya mitoticheskikh figur v rezul'tate ikh vzaimodeistviya (Morphological Changes of Mitotic Figures as a Result of Their Interaction). — In: Sbornik rabot po mitogenezu i teorii biologicheskogo polya. Izdatel'stvo Akademii Nauk SSSR. 1947.

N.A. Grigoryan

THE BIRTH OF ELECTROCARDIOGRAPHY IN RUSSIA

At the end of the nineteenth century, almost one hundred years after Galvani's discovery, an event occurred in the development of electrophysiology which made possible the birth of a new branch of science, electrocardiography. In 1889, the English scientist A. Waller published a paper[1]* in which the first human electrocardiograms were described. Waller succeeded in tapping the cardiac currents of the intact organism. By attaching electrodes and a capillary electrometer to the arms and legs of a person in a certain way, he obtained the first tracing of currents of the beating human heart. Waller's tracings have nothing in common with what is now called an electrocardiogram. They are of historical interest only. Waller's great service consists solely in the fact that he proved in principle that it was possible to obtain information on the heart from electrical effects. Therefore, A.F. Samoilov was perfectly justified in calling him the "father of electrocardiography."

Despite the fact that at the present time there is extensive literature in the field of electrocardiography and the number of articles and monographs is increasing daily in the Soviet Union as well as in other countries, there are no studies on the history of this science, not even articles of a review character. We refer to the history of electrocardiography from the aspect of its physiological and clinical significance. Nor are there studies of the life or activity of the scientists, the creators of present day electrocardiography. The literature concerning W.Einthoven is limited to short articles written on his being awarded the Nobel Prize[2] and by pathologists. Wenckebach's[3] articles and the reminiscences of A.F. Samoilov[4] are particularly valuable. Only in 1955 was a small book by S. Hoogerwerf[5] published in Dutch dedicated to Einthoven. The literature on the founder of electrocardiography in Russia, A.F. Samoilov, is also limited to separate articles written for a special edition, that of the Kazan' Medical Journal dedicated to the memory of Samoilov (1931).

The founder of present day clinical electrocardiography, the Dutch physicist Willem Einthoven, concerned himself extensively with the physical problems of physiology and made a careful study of anatomy, histology, and optics. Dubois Reymond, Helmholtz, and Engelman exerted a considerable influence on the formulation of Einthoven's scientific views and the direction of his studies. During his life Einthoven published little. However, all his papers are characterized by completeness and accuracy. Einthoven's studies were concerned mainly with electrophysiology. Even at the very beginning of his independent scientific activity Einthoven began to record and study the electrical phenomena of muscles and nerves, particularly the electric currents of the heart.

* [For this and other footnotes appearing in this text, see "References" at the end of this paper.]

The success of work in this direction depends a great deal on the level of technology. In Einthoven's time the capillary electrometer devised by the French physicist Lippman was used extensively in electrophysiological research. However, the curves of cardiac currents obtained with the capillary electrometer required modification. Einthoven spent much time in correcting these curves. It was clear to him that the capillary electrometer was not the instrument that electrophysiology needed. In 1903, after long and arduous research, Einthoven succeeded in constructing a new instrument, a string galvanometer, the use of which was the turning point in the difficult and complicated history of electrophysiology. In his work "Galvanometric Recording of the Human Electrocardiogram,"[6] Einthoven for the first time described the construction of his string galvanometer and the curve of human cardiac currents obtained with it. He wrote: " . . . I have invented an instrument which satisfies all the requirements, and is particularly suitable for recording the human electrocardiogram directly in almost exact proportion.

The essential part of this instrument — the string galvanometer — is a silvered quartz filament which is stretched like a string in a strong magnetic field. When electricity passes through this quartz filament it moves, and this movement, like the movement of the capillary electrometer, can be seen and photographed if it is considerably magnified. It is possible to regulate the sensitivity of the galvanometer very carefully without great tension or slackening of the string."[7]

The creation of the string galvanometer was the turning point which physiologists had awaited for many years. The first clinical use of the string galvanometer was in the examination of patients at the Leyden teaching hospital's physiology laboratory. The hospital was more than one kilometer from the laboratory. For the purpose of taking the electrocardiogram both buildings were connected by electric wires. Einthoven wrote, "a tracing recorded by such a method at a distance could rightfully be called a 'telecardiogram'"[8]. This solution of the problem was not without basic defects, because numerous lighting, telephone, trolley car, and other wires could influence those connecting the laboratory with the clinic and distort the tracing of the cardiac currents. Einthoven spent much money and effort in overcoming all the obstacles he encountered but a drawback to his solution was that it became very difficult to record the pulse and cardiac impulse, if not entirely impossible.

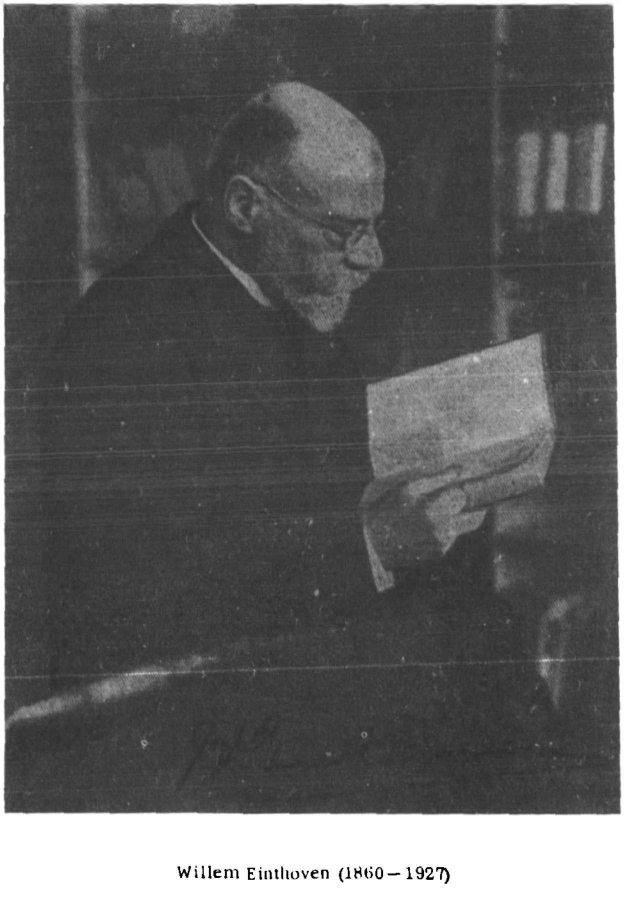
Afterwards, Samoilov used a different method and made all the studies in his own clinic.

After Einthoven had perfected the method of string galvanometry he continued working on the improvement of his instrument so as to facilitate its use on regular and field hospitals. Einthoven did an exceptional amount to have this method mastered by rank and file physicians. He created not only the method itself but also the theory of electrocardiography; he worked for many years on the interpretation of the normal record of cardiac currents as a solid basis for understanding cardiac pathology. His explanation of each wave complex in the curve obtained assisted in revealing the mechanism of the electrocardiogram. All the methods proposed by Einthoven, namely, standardization, type of electrodes, tapping from three points, and even the terminology will retain their importance in electrocardiography for a long time if not forever.

Einthoven's contributions in the field of electrocardiography were highly regarded. In 1924, he was awarded the Nobel Prize in Medicine and Physiology for his discovery of the mechanism of the electrocardiogram. Einthoven concluded his speech on obtaining the Nobel Prize with the following words: "the new chapter in the scientific study of heart disease has not been written by a single individual but is the work of those many talented persons who made their scientific research irrespective of political boundaries. These persons throughout the world devoted their energies to an ideal, the development of science, which will ultimately benefit suffering mankind."[9]

Under the influence of Einthoven's work electrocardiographic studies began to be made shortly after by scientists of many countries.

The study of the 50-year-old history of electrocardiography shows how closely interrelated are the interests of physiology and medicine, the laboratory and clinic on the one hand, and physics and physiology on the other. At the present time, the role of physics and engineering in medical research is very great. The use of the latest technique implies a basic comprehensive knowledge of physics, particularly electricity and optics.



Willem Einthoven (1860—1927)

An attempt to combine physiological and clinical problems was made at the end of the nineteenth century. Russia and the United States were the pioneers in this field.

Characteristic of Russian medicine at the end of the nineteenth century was an attempt to utilize new laboratory methods of clinical investigation and to attach to the clinics laboratories in which experiments could be performed on animals and sometimes on man. S.P. Botkin spoke about this in his talk,[10] "General Principles of Clinical Medicine." I.M. Sechenov studied medicine together with Botkin at Moscow University and attempted to devise efficient methods of treatment based on physiology. Sechenov wrote, "the phenomena of nerve stimulation by direct and interrupted current, phenomena which should constitute the basis for efficient electrotherapy and the starting point for its future development deserve special attention."[1] "... a partnership with equal rights and mutual benefits has been created between theoretical and practical medicine," said Pavlov, "which is the basis of progress of medical science."[12]

The founder of electrocardiography in the Soviet Union, Aleksandr Filippovich Samoilov, was firmly convinced from the very beginning that electrocardiography should become the subject of the physician's study, that it should be included in clinical medicine where extensive research would be done on it. In this respect, Samoilov's report to the Annual Convention of the Moscow Society of Internal Medicine on 30 January 1908 was very characteristic.[13] Incidentally, like many other articles by Samoilov in the field of electrocardiography, this paper has now become a bibliographic rarity; we shall therefore analyze it in further detail, particularly since it was essentially the first general work by Samoilov on this subject.

First of all, we should like to call attention to the fact that Samoilov considered it necessary that the report be specifically for internists. In the early stages of electrocardiography, when in many countries of the world, with the exception of Holland and Russia, essentially very few had any idea of the string galvanometer and its inventor, Samoilov's talk was striking evidence of his clear understanding of electrophysiological problems, of the advent of a new stage in the development of medicine and physiology.

At the beginning of his report Samoilov said that "animal electricity ... has taken the true road of current scientific problems", that " ... electrophysiology has become more modest; the problems which it has set before itself are at present of a specifically defined, real nature. One of these problems is the study of the electric currents of the heart and the elaboration of methods of recording them graphically in the form of a curve."[14] Samoilov said later, "one can hardly doubt that electrocardiograms play a great part in the diagnosis of cardiac diseases."[15]

Samoilov gave a description of the first case of recording the currents of the sick heart (before that he had recorded cardiac currents in his laboratory workers, healthy persons.) "A string galvanometer," said Samoilov in his report, "was required for the laboratory, not for examining patients. I recorded the electrocardiogram of a normal human heart only to check the quality of the instrument regarding an absolutely defined, comparatively difficult task presented to it. However, when I had mastered the technique of using the instrument, at Professor A.N. Kazem-Bek's request, I agreed to record the cardiac current tracing of a sick woman sent to the laboratory from the clinic. I was not aware of the nature of her cardiac disease and certainly did not examine her, because I have not engaged in medical practice since I was a student. I directed her cardiac currents through both hands to the galvanometer and exposed the electrocardiogram. After a minute, while developing the tracing in the dark room, I noticed a very marked deviation of the tracing from the normal type. It was clear that the part of the electrocardiogram which corresponded to the atrium was working under greater strain than usual for a healthy person. It had been ascertained by ordinary methods that the patient had a constriction of the left atrioventricular orifice. Thus, my diagnosis, essentially the same as that of the clinician but based on electrocardiographic data, determined the nature of the disease from a different aspect, the aspect of the strength of atrial activity."[16]

Then Samoilov spoke of the significance of the new physiological method in the study of cardiac disease, of caution in its application, and of the fact that physicians with good physical and physiological training should come to the clinic. Samoilov said, "The clinic is a place which is, so to speak, in the position of a person who is about to receive a great benefit and must utilize this benefit to its utmost.

"... The application of cardiac currents to pathological cases is not yet in general practical use. Evidently this problem will remain for a long time the subject of scientific investigation solely, and of careful and cautious investigation at that; caution is required here, particularly because of the complexity and fineness of the examination method, as the more complicated a method the greater the possibility of error."[17] Samoilov concluded his talk with the words that future studies in the field of electrocardiography "no matter what form they take would not only satisfy our scientific curiosity but would be of benefit and aid to that delicate and suffering organ of present-day humanity, the heart."[18]

Only in the 1920's however, through the achievements of the October Revolution, did it become possible to carry out what men of medicine and physiology had dreamed of and striven for, namely, "a legal union" between physiology and medicine. At present, science, even the so-called "pure science", could be applied to practice and thus serve the interests of society. The example of electrophysiology is particularly significant in this respect.

Samoilov was one of the scientists who, during the first few years of the Soviet regime, placed electrophysiology at the service of medicine at the first opportunity, organizing his excellently equipped electrocardiographic laboratory at the Kazan Institute for Advanced Training of Physicians. He wrote: "The attempt to bring into the clinic a theoretician who would study the functions of a sick person with the same care and with the same scientific accuracy with which physiology is studied on a human subject, has brought to life a whole series of medical institutions of a particular type." Further, "... under the conditions of life in Russia this idea can be realized primarily by incorporating theoretical laboratories into our clinical institutes. The clinical institutes are not only therapeutic institutions—hospitals—but chiefly institutions which serve educational and scientific goals...

"One may express the desire of inculcating in the Kazan Institute the healthy promising trend in medicine which sets itself the aim of introducing the spirit of laboratory investigation into the clinic..."[19]

The importance and timeliness of Samoilov's article can be judged from the fact that twenty-three years later in an article by Academician

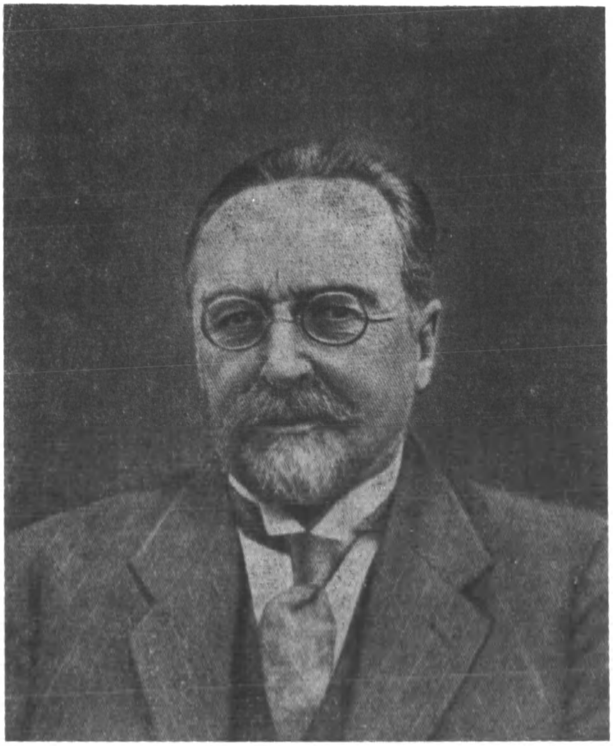
K.M. Bykov[20], and thirty-five years later in an article by I.V. Davydovskii[21] the same theme was sounded.

In 1904, while professor at Kazan University, Samoilov met W. Einthoven in Brussels at the Sixth International Congress of Physiologists. His acquaintance with Einthoven and his string galvanometer was responsible for the subjects of Samoilov's research for many years. Three important factors in Samoilov's life contributed to the creation of physiological and clinical electrocardiography in Russia: 1) work in Pavlov's physiology laboratory, Pavlov's idea that physiology and medicine are inseparable and that physiology should serve medicine and become its basic; 2) work in Sechenov's laboratory and Sechenov's idea of a close connection between physiology and physics, of the accuracy of the physiological experiment, and a deep understanding of physics and mathematics; 3) W.Einthoven's influence and the progress of technology. One can hardly doubt that the combination of these three factors specifically led Samoilov to the important discoveries through which he became well-known as a prominent physiologist even during his lifetime far outside his own country.

A.F. Samoilov acquired the first string galvanometer and set it up in the physiology laboratory of the Faculty of Physics and Mathematics of Kazan University in 1906. Samoilov wrote more than twenty papers on electrocardiography, the majority of which were written between 1906 and 1917 and were published chiefly in German journals. These articles were not translated into Russian. Apart from his published papers, Samoilov also left a number of manuscripts. In the last few years of his life he attempted to generalize on his electrocardiographic studies. He planned to write a monograph, but unfortunately his unexpected and premature death prevented the realization of this plan. Samoilov's unfinished manuscript, "Electrocardiography," is permeated from beginning to end with the single thought that electrophysiology should become a vital science and should assist in the solution of many unsolved problems of medicine. With great satisfaction, Samoilov wrote the following, "... We are witnesses of the fact that the electrophysiological method has ceased to be the source of academic theorizing concerning the nature of the excitation process as it had been at the time of Dubois Reymond, and is now being used to assist in resolving the inherent problems of physiology. Gradually, the electrophysiological method is penetrating clinical medicine, specifically the clinics of nervous, muscular, and cardiac diseases."[22]

Samoilov defines electrocardiography in the following way: "Electrocardiography is a part of electrophysiology, a division concerned with the phenomena of the development and course of electricity in the animal organism. Thus, the study of animal electricity is frequently called electrocardiography."[23] Samoilov wrote that the tracing of the electric current recorded by the filament of the string galvanometer makes it possible to reproduce the spread of excitation in the heart as though we had seen it with our own eyes running from the sinus node to the ventricle. Finally, we have another definition of electrocardiography by Samoilov: "Electrocardiography, the study of the recording and reading of the cardiac current tracing, the electrocardiogram, is a powerful tool for evaluating the normal and pathological course of excitation through the heart."[24]

Though Samoilov was interested chiefly in the scientific development of electrocardiography, the intention to use it in practice is evident even from his first few papers. Thus, his paper of 1896,[25] which is chiefly of a methodical nature, Samoilov already spoke of the benefit of this new method for the clinic of cardiac diseases.



Aleksandr Filippovich Samoilov (1867 – 1930)

In 1906, Samoilov published his first paper on electrocardiography[26] with the use of the perfected capillary electrometer. In this paper he showed that the form of his electric wave of cardiac action currents is connected with the continuous conduction of excitation from the atrium to the ventricle, that the heart muscle has a refractory period, and that change in the shape of the curve of cardiac electric currents depends on the effect of the environment on the preparation (for example the effect of temperature). From the frog heart Samoilov obtained a biphasic curve, thus indicating that oppositely directed phases are not the rule. By changing the conduction conditions in various parts of the ventricle Samoilov obtained corresponding changes in the electrical tracing. Samoilov regarded the shape of the electrocardiogram as the result of changes in electrical potentials under the leads.

In 1908, Samoilov gave a brief description of the properties of the string galvanometer and methods of its application.[27] On this instrument he repeated the experiments conducted with the capillary electrometer and confirmed his previous data. For the first time in this paper Samoilov showed the connection between the electrocardiographic changes and respiratory movements.

Investigators engaged in recording cardiac action currents could not determine the influence of respiration on the electrocardiogram. F. Kraus and G. Nicolai found that vigorous respiration leaves the electrocardiogram unchanged. Samoilov wrote, "I myself have different data on this subject. Even before Einthoven designed the string galvanometer, when the human electrocardiogram could be studied only by means of the capillary electrometer, I was able to observe in a lecture demonstration of human cardiac action currents that respiration, specifically vigorous respiration, exerts a very strong influence on the mercury level. I noted specifically that during the phase of expiration the level becomes higher and, by using forced expiration and increasing the small respiratory excursions the demonstration was made more distinct. With the string galvanometer the respiratory effect can be proved even more convincingly."[28] Einthoven called attention to this aspect of Samoilov's work.

In the same work Samoilov described combined electrocardiograms of two laboratory workers. Here it was found that the electrocardiogram of each person has a constant form with the sole difference being in the height of the "T" wave. According to the figurative expression of Samoilov, "When we shake hands, our own cardiac currents pass through both bodies." The galvanometer can show the respective spatial relationships of both persons." Referring to M.Kramer's data on the possibility of determining the presence of a fetal heart in a pregnant woman, Samoilov wrote that the electrocardiogram can confirm the clinical diagnosis in gynecology. It can be used not only to establish the presence of twins but also the interrelationship of both fetuses. Samoilov drew the important conclusion that "each normal electrocardiogram of a single heart should be regarded similarly as a combined one. Because under normal conditions our right and left hearts work synchronously, and the combined nature of the resultant action currents remains undetected."[29]

In 1909 Samoilov published a paper[30] which is mentioned in almost all the major works on electrocardiography. He described the electrocardiographic changes in three patients suffering from mitral insufficiency. He noted that electrocardiography makes it possible to delve into the pathology of the atria, specifically that portion of the heart which is difficult to study by ordinary clinical methods of examination. Samoilov described the changes in the atrial "P" wave. He ascribed great importance to this wave, calling it the "real pearl of the electrocardiogram"; in two cases Samoilov noted an enlargment of the P wave; in one case it was absent from a patient with a severe form of stenosis, coinciding with a deterioration in the patient's condition. Samoilov wrote, "What I should like to call attention to is the fact that in far-advanced cases of mitral stenosis the originally enlarged P wave of the electrocardiogram can gradually disappear indicating progression of the disease, a sign of atrial insufficiency."[31] Samoilov explained the enlargement of the P wave by the fact that in mitral stenosis, as a result of difficulty in the movement of blood, the atrium offers resistance and increases its activity as a compensation. Quite recently, Professor P. White, President of the International Society of Cardiologists, recalled this work in his lecture dedicated to the memory of Einthoven. Speaking about his first meeting with Einthoven and his initial work on the string galvanometer, White noted: "At this time, in Russia, his close friend and colleague, Professor Samoilov from Kazan, showed an enlargement of the P wave on the electrocardiogram caused by the enlargement of the left atrium in mitral stenosis."[32] This was Samoilov's first attempt to confirm a clinical diagnosis by the electrocardiographic method, thereby placing electrocardiography at the service of clinical medicine.

In 1910 Samoilov's renowned article appeared, "A Further Contribution to Electrophysiology of the Heart."[33] In this article Samoilov made an attempt to generalize on some of the results of electrocardiography and gave his conception of the electrocardiogram and the significance of its waves. "By obtaining a large number of electrical tracings," he wrote, "they can be seen to actually be variants of the same tracing. Now you will learn that the situation as a whole deals with different combinations of certain parts of the tracing. Einthoven's great service is his discovery of these parts of the tracing and his explanation of their general significance. Einthoven's P, R, and T waves always make up the tracing of cardiac currents."[34] Samoilov went on to say that the elevation of the P wave is a constant part of the electrocardiogram going in a single direction and is never absent, whereas the T wave frequently changes not only shape but also direction. Even for the electrocardiograms of mammals the T wave is the most sensitive part of the tracing of cardiac action currents. Mechanical and electrical reactions of the heart proceed in parallel; in certain instances, judgements can be made about one on the basis of the other. Samoilov expressed the very important idea that in the heart, along with electrical phenomena resulting from the spread of excitation through the heart muscle, so other phenomena may arise as the expression of metabolic processes. Specifically, Samoilov was inclined to ascribe the final portion of the electrocardiogram, the T wave, to metabolism.

Then, Samoilov gave a detailed description of the change in the waves under the influence of different conditions. He wrote, "if a whole series of electrocardiograms is taken with artificial stimulation, some interesting observations can always be made which, incidentally, for the most part illustrate the difference between the nature of the P and T wave. The more the electrocardiogram is studied under different conditions, the more opportunities there are of observing that the P and T waves do not change similarly under the influence of the same action; or that a change in one wave may not be accompanied by a change in the other. I found the effect of increased respiration on the human electrocardiogram was an appreciable change in the size of P, while T maintained its size. Still more frequently exactly the opposite occurs, where the T changes and the P remains unchanged. The form of the electrocardiograms in various subjects is distinguished chiefly by the T wave, which is the most sensitive part of the cardiac current tracing."[35]

In the same paper Samoilov touched on a problem to which a number of serious studies were subsequently devoted — the effect of vagal stimulation on the electrocardiogram. As a result of numerous experiments with vagal stimulation of the heart of cats and dogs Samoilov concluded that the effect of stimulation of the vagus nerve is expressed on the electrocardiogram by diminution or by change of direction of the T wave. The P wave remains unchanged. The fact that the T is the most sensitive part of the electrocardiogram has been confirmed specifically through vagal stimulation.

In an investigation in 1914[36] Samoilov showed that stimulation of the vagus nerve causes the following changes in the ventriculogram: the T wave, which before stimulation is as positive as the R wave, decreases in size and, as a rule, becomes negative. Samoilov wrote that his data on the vagal change of the P wave was shortly afterwards confirmed and proved by other authors, particularly D. Dale and G. Mines.[37]

"Without a hypothesis as to the form of the cardiac action currents, however," Samoilov wrote, "it is impossible to explain the vagal alteration of the electrocardiogram."[38] Here Samoilov came close to understanding the mechanism of the origin of the electrocardiogram wave. He turned to authors who had taken up this problem before him. The first investigators of cardiac action currents had come to the conclusion, by means of a differential rheotome, that the ventricle produces an action current consisting of two opposite phases like an individual skeletal muscle. In the first papers a capillary electrometer obtained tracings of the action currents of the ventricles showing two opposite phases. Einthoven, making his studies on the hearts of mammals and particularly of man, first by means of a capillary electrometer and then with a string galvanometer showed absolute confidence that the ventricular currents consist chiefly of two waves going in the same direction. A.F. Samoilov wrote: "I have shown, first by means of the capillary electrometer (Beiträge zur Elektrophysiologie des Herzens, 1906) that the ventricle of the frog when tapped from two surface points can produce a tracing with two waves in the same direction, like the R and T waves of Einthoven's electrocardiogram of the mammal. Gotch came to the same conclusion at almost the same time.

"As I noted later (1910) with artificial stimulation of the frog ventricle and change in the sites of stimulation (base or apex of the heart) there is a change in the sign of the R wave only; the direction of the T remained unchanged. Therefore, the form of the cardiac action currents depends on the spread of the excitation wave."[39]

In 1927, Samoilov in cooperation with his students E. Mel'nikova, A.Panina and I. Sokolova studied the effect of vagal stimulation by muscarine on the cardiac action currents.[40] He wanted to compare the effect of the vagus substance present in Ringer's solution with the effect of vagal stimulation proper on the electrical phenomena of the heart in order to find a basis for the study of the nature of the vagus nerve. Samoilov concluded that the changes in the form of frog ventricle action currents caused by stimulation of the vagus with muscarine are very similar to those which occur with electrical stimulation of the vagus nerve. This result can be understood in the sense that the effect of the fluid is probably of the same nature as the inhibitory effect of the vagus itself.

Finally, in 1917 and 1923 Samoilov published studies associated with some interesting phenomena on stimulation of the vagus nerve of the heart of a turtle.[41] In these experiments Samoilov attempted to explain the Gaskell phenomenon.

In 1887 the English physiologist W. Gaskell, using the heart of a turtle which had been inactive for a long time, observed an increase in the resting current (positive wave) on stimulation of the vagus nerve.[42] Gaskell ascribed great importance in principle to this phenomenon, because the vagus nerve acted on muscle cells in the direction opposite to that of motor nerves on muscles which produce a negative wave in the resting current when stimulated. In the same year Gotch used the capillary electrometer; later (in 1910) Einthoven used a string galvanometer but neither was able to confirm the Gaskell phenomenon. In 1913 Samoilov and M.I. Sergeev, together repeated Gaskell's experiment on a tortoise, using the string galvanometer. However, in 1916 Einthoven again came out with a denial of Gaskell's phenomenon. This constituted the basis for a special study by Samoilov on the subject. "Our tracings," Samoilov wrote, "leave no doubt that the phenomenon of the positive wave on stimulation of the vagus nerve in Gaskell's experiment is of an absolutely definite nature, has a definite course and represents a phenomenon which is reproducible with no greater loss constancy than many other physiological phenomena . . . It seemed to me that the result of Gaskell's experiment may be considered, at least from the factual aspect, as unquestionably correct. If I now return to our previous experiments and subsequently describe them in greater detail, this is because most recently a new large-scale study has been made by W. Einthoven and A. Rademaker (1916) concerning Gaskell's experiment, which brings to naught everything which Gaskell affirmed."[43]

Einthoven and Rademaker[44] believed that the entire Gaskell phenomenon was based on a defect in Gaskell's method, undetected by Gaskell himself as well as by others who later repeated his experiment. They believed that stimulation of the vagus nerve leads to contraction of the turtle's lungs. On contraction the lungs draw on the suspended right heart, and the traction of the muscle tissue always increases the resting current. Einthoven and Rademaker believed that the effect of inhibitory nervous activity is no longer significant. Samoilov wrote: "The question we are dealing with is very important in principle, because it concerns a certain intrinsic aspect of the phenomenon of inhibition, a phenomenon whose part in the group of vital processes proves to be as all-embracing as the role of excitation processes."[45] The differences in the views of such authoritative investigators as Gaskell and Einthoven require further research, and Samoilov undertook a number of new experiments with his pupil V.I. Bashmakov. He concluded that "Gaskell had observed and deduced correctly: the vagus nerve on stimulation produces a change in the resting atrial muscle of the turtle, and this is accompanied by an increase in the initial electric resting current. Einthoven and Rademaker observed and described correctly the interesting phenomenon of contraction of the lungs. Superficially, Gaskell's phenomenon may appear to be distorted but essentially this has no bearing on the matter."[46]

Samoilov returned to this problem in 1923; he performed some additional experiments like the previous ones in cooperation with M.I. Sergeev and V.I. Bashmakov, and he published an article in Pflüger's Archives. After receiving an excerpt of this article Einthoven, in a letter of October 2, 1923, wrote the following to Samoilov: "Although your work did not convince me of the positive wave, I read it through with great interest. I believe that you overlooked a certain fact; namely, that a slight change in the position of the preparation can cause an appreciable change in the demarcation current."[47] After Samoilov's death in 1931 an article was published in the Kazan Medical Journal by Danilov,[48] a student of Samoilov, who had taken up this problem at Samoilov's request and confirmed Gaskell's phenomenon.

Academician L.A. Orbeli believed that the intrinsic effector mechanism of the centrifugal nerves of the heart, and therefore of sympathetic and parasympathetic hormones, should be regarded as essentially a trophic mechanism which changes the basic functional characteristics of the tissue. Referring to Gaskell's phenomenon Orbeli said: "Only Samoilov succeeded in finding a method enabling him to accept the positive wave in the heart muscle as induced by vagal stimulation. However, for some reason these data of Samoilov are not generally recognized."[49]

In 1928, Samoilov's[50] article was published, and was of great theoretical and practical interest. In 1910 Samoilov had obtained a monophasic curve through chance injury of the heart muscle during an experiment. This made the use of electrocardiography possible in principle, for recording the pathological changes in the myocardium. At the end of his life Samoilov made a systematic experimental study of this problem. V.F. Zelenin, who had begun this study of electrocardiography almost simultaneously with Samoilov, albeit with a definite clinical bent, considered these works by Samoilov highly. He wrote: "The data which he (Samoilov) obtained, the prototype of which is the so-called monophasic curve, constituted the basis for the explanation of the electrocardiographic changes in myocardiac infarction".[51]

In a comparatively simple preparation, a "bridge," obtained through partial resection of the ventricle, Samoilov studied all the aspects of the conduction of excitation and came to important theoretical conclusions. He believed that the "bridge" reproduces the atrioventricular system and can serve as an excellent object for the study of all abnormalities in conduction After studying the electrograms obtained, Samoilov concluded that the excitation process undergoes certain changes in passing through the bridge similar to those observed by N.E. Vvedenskii in a nerve fiber. Samoilov likened Vvedenskii's parabiosis to the conduction disorders occurring in the bridge and based his interpretation of both complete and partial inhibition in the bridge on this conception. "This phenomenon," Samoilov wrote, "seems to us like parabiosis in nerves, which was so carefully studied and thoroughly described by the Russian investigator, N. Vvedenskii."[52] In the bridge Samoilov observed the phenomenon of partial atrioventricular block which is today known clinically as the Wenckebach-Samoilov period and is expressed in a gradual increase in the A-V interval. When this increase reaches a certain limit, the A-V interval following the blocked contraction is normal. Samoilov explained the mechanism of the A-V block by the state of permanent standing excitation in the bridge. Other authors believed that the reason for the block was the narrowness or tortuosity of the path connecting the atrium with the ventricle (Gaskell, Scramlick and others) or a reduction in the quantity of glycogen in the A-V node (Lewis).

This work by Samoilov was continued by his students: M.A. Kiselev, I.L. Kan, E.I. Borisova, V.S. Rusinov and others. Borisova and Rusinov wrote the following: "Based on Samoilov's, Kiselev's, Pavlov's and our own work, it may be stated very definitely that the functional condition of the bridge in the partially resected ventricle is a state of developing parabiosis."[53]

Samoilov always eagerly assisted clinicians, particularly in cases when the clinical picture of the disease was not entirely clear and required electrocardiographic examination of the patient. In this respect an article[54] written jointly with Professor M. N. Cheboksarov, Director of the Clinic of the Faculty of Internal Medicine of Kazan University, is characteristic. It was concerned with patients in whom a complete A-V block was combined with a disorder of atrial activity. In the article two interesting cases are presented: In one patient there was a very rare combination (in the literature only one similar case described by Wenckebach in 1914 was known) of a congenital developmental defect of the heart in the form of a patent ductus arteriosus and complete heart block; in another patient, an A-V block was also diagnosed clinically, but a disorder of atrial activity in the form of the establishment of the Mines-Lewis circus rhythm, that is, atrial fibrillation, could not be diagnosed clinically, because the irregular pulse, the pulsus irregularis perpetuus, characteristic of atrial fibrillation was absent in this patient owing to the complete A-V block. The authors wrote, "only by means of the electrocardiogram were we able to establish that in patient N. there was a very rare combination of a complete heart block and atrial fibrillation."[55]

In 1930, in cooperation with A.Z. Chernov, Samoilov's work[56] in the electrocardiographic division of the Institute of Occupational Diseases imeni Obukh in Moscow was closely related to the so-called circus rhythm first described by G. Mines.[57] In an experiment on the frog heart on cessation of artificial stimulation Mines noticed a rapid, uniform rhythm. The atrium appeared to have caused the ventricle to beat, or vice-versa, as it were, thereby creating an interrelated rhythm. Subsequently, this rhythm was described by P. White, A. Drury, D. Scherf and other authors. In the article by Samoilov and Chernov a case of "reciprocal rhythm" in human beings is presented.

In 1930 the article, "Circus Rhythm," was published summarizing the studies of Samoilov in the field of electrocardiography; in it some theoretical and practical problems of cardiac physiology and pathology were solved. The mechanism of a number of complicated nerve-reflex reactions was revealed. Samoilov begins his article with the history of the subject. The expression "circus rhythm" was originated by the English physiologist Mines. This rhythm was created and maintained by the movement of excitation over a closed pathway. Circus rhythm was first induced by the American biologist Meyer on the annular muscle of the umbrella of a medusa. Meyers' experiment was repeated in a very objective form by a student of Samoilov, I.A. Vetokhin, on the annular muscle of a medusa at the Murmansk Biological Station in 1926 — 1927. In one of Vetokhin's experiments on a ring 60 cm in length the excitation ran 5½ hours, traversing the ring 15 times a minute; therefore, the entire route travelled by the wave in this time was equal to 5 km. Samoilov wrote, "I.A. Vetokhin's excellent experiment is evidently a most elementary, uncomplicated and particularly valuable form of circus rhythm."[58] Mines' circus rhythm was different from other rhythmical movements (alternation of day and night, succession of seasons, etc.), Samoilov said, in the fact that it is not mass which moves in it but a process, specifically an excitation process.

It was found that the animal heart is also a reliable subject for demonstrating circus rhythm. Mines could always demonstrate circus rhythm at will on the turtle heart. "My co-worker V.R. Bashmakov and I have repeatedly performed this experiment together. It is very impressive. The contraction evidently passes from one half of the ventricle to the other, and then, after a definite pause is redirected to the atria and after another pause, back to the ventricle, etc.

"This circular movement once produced in the turtle heart preparation can continue a very long time."[59]

Mines did not describe the movements of the separate parts of the heart during circus rhythm. Samoilov believed that for the analysis of the phenomenon of circus rhythm it is essential to record the electrical effect of the contracting heart muscle. He recorded the electrogram of four divisions of the turtle heart (four pairs of electrodes were applied to two atria and two parts of a partially resected ventricle). He wrote: "The phenomenon of circus rhythm of the heart . . . may appear to be a curious, albeit interesting fact at first glance or, at best, a fact of only theoretical importance. However, its importance is not only theoretical. Anyone acquainted with the physiology and pathology of cardiac rhythm knows that the forms of arrhythmia produced artificially in the experiment can almost always be recognized in patients in pathological cases, and vice-versa: the cardiac rhythm disorders observed in man can be produced experimentally on animals. Mines predicted with great perspicacity that the phenomena of circus rhythm would help to explain the surprising phenomenon known in the heart clinic as 'atrial fibrillation'"[60] and further: "the question of the origin of atrial fibrillation has proved to be a very complicated one and has traversed a long and difficult route, but today atrial fibrillation and the so-called 'atrial flutter' are with sound reason based on the fact that there is interference with the normal course of excitation in the atrium, and a circus rhythm is established in place of it."[61]

Based on the experiments of Mines, Meyer, Lewis, Vetokhin, and his own, Samoilov concluded that "in the medusa, in the heart of the fish and especially in the heart of the turtle and in the atrium of dog and man a circus motion is established under certain conditions."[62] Samoilov believed that these examples far from exhaust the possibilities of a continuous course of excitation over a closed pathway. "The area of the circus rhythm is boundless;" he wrote, "a continuous course of excitation is required over the same circus pathway for each moment of our existence."[63]

Samoilov analyzed in detail the mechanism of a number of simple and complicated reflex movements; he based his investigation on Rich's factual material of the electrophysiological study of nerve reflex phenomena which he had obtained in cooperation with M.A. Kiselev. This made it possible for Samoilov to draw a number of conclusions which threw new light on the whole study of the reflex, of particularly great importance to the development of present-day science. He described the typical "closed reflex." This is what he said: "I take a pencil in my hand to write. During the entire time I am holding the pencil in my hand impulses are going from the spinal cord to the muscles which participate in the act of contraction for holding the pencil. The muscles function not only as organs of work; they are also a distinctive and, rightfully, primitive sense organ of our bodies. Among the muscle fibers there are specialized sensory structures from which sensory fibers carry impulses to the spinal cord. Muscle contraction produces a mechanical stimulation of the sensory nerve endings in muscle; the signals coming from here control and direct the muscle innervation as a reflex and thereby guide the muscle contraction. All the time I am holding the pencil impulses follow one another from the sensory nerve endings in the muscles to the nerves in the spinal cord and from here over the motor nerves to the muscles, and from them, by means of mechanical stimulation, back to the sensory endings in the muscles, and so on, always in the same order, carrying out the phenomenon **which I suggest calling the closed reflex"** (author's stress, N.G.).[64] Further: "the excitation ring which we have represented for the closed reflex is certainly only a simplified scheme of the mechanism which is triggered in cases of holding an object with the hand, because in our case the influence of the will is felt in the reflex process. Pathways extend centripetally toward the brain, from the spinal cord over collaterals from the sensory nerve and from here fibers go centrifugally to the spinal cord. Under the guidance of kinesthesia the mechanism of the closed reflex ring can be controlled at will and directed toward accomplishing its purpose. However, the manner in which the brain participates in the spinal cord reflex mechanism is also a closed circular pathway ... in all cases in which a definite position of the body is maintained circular pathways are formed from nervous and muscular elements over which excitation impulses inevitably travel: the impulses which reach the muscles produce the necessary degree of contraction, the muscle itself regulating these impulses through those produced by the stimulation of its sensory nerve endings. Change in position is accompanied by the movement of excitation from circular pathways of the closed reflex of the former posture to others."[65]

There is no difference in principle between the various instances; the only difference is in the fact that excitation does not always spread by means of direct contact, and sometimes it is through a system consisting of dead and living parts. Samoilov concluded his article, "Circus Rhythm," with a broad philosophical generalization and the hope that the phenomenon which he had discovered would play a role of some importance in the development of science. He wrote, "We began this article about circus rhythm with a presentation of experiments on a muscular ring of a medusa. This phenomenon, surprising and interesting, nevertheless was absolutely unrelated to anything. However, the entire study of natural history tells us that no phenomenon in nature can occur apart, isolated from others: all of nature is a single whole, and we see only partial manifestations of it.

"The circus rhythm of the medusa ring showed us one of the characteristics, one of the capacities of the living machine. While this characteristic is found under absolutely exceptional artificial conditions, nevertheless, its significance was undoubted. It may be expected that the circus rhythm will let itself be known, will manifest itself in other subjects, in other varieties, in other forms and combinations."[66] Samoilov expressed the idea that in some cases appropriate mechanisms may be placed at points of interruption for the continuous spread of excitation — a transmission apparatus for the uninterrupted transmission of excitation around the ring. A unique ring with points of subsidence and creation of excitation would be gained.

Extensive excerpts from Samoilov's article are presented here because certain principles and thoughts expanding the concept of the reflex and the reflex arc were formulated in them. The expression "circus rhythm" was introduced by Mines; the phenomenon of the circus rhythm was obtained for the first time by Meyer; however, the credit for electrophysiological study of this phenomenon goes to Samoilov. He extended the phenomenon of the circus rhythm to complex reflex processes, developed the study of the reflex expressed in the formulation of a new concept in physiology, those of the "closed reflex" and of the "closed reflex arc."

Samoilov's first statements about circus rhythm and the closed reflex were in 1925 – 1928. During these years Samoilov studied the nature of the transmission of excitatory and inhibitory processes. Investigation led him to conclude that the transmission of nervous processes from one cell to another is accomplished by means of the production of some kind of chemical substance. In 1927, in his report in Berlin during the week of Soviet science in Germany, Samoilov said: "At this very moment, as I am presenting this report, to which those present are attentively listening, and I, in turn, am perceiving the effect of my words, the whole auditorium appears to be filled with complex closed circuits including my listeners and myself, as well as the environment separating us. All these components appear as a single organism, so to speak, in which one cell acts on another by means of chemical stimulation,"[67] Samoilov concluded his report with the thought that on deeper analysis the very specialized scientific problems lead to conclusions of a general nature, to conclusions attesting to the unity of life.

In a report to Kazan physicians in 1928, dedicated to the 300th anniversary of publication of W. Harvey's work "De motu cordis", Samoilov said that Harvey not only discovered circulation; by his discovery he indicated the existence of phenomena in the animal organism occurring over a closed pathway. "Now I should like to call attention," said Samoilov, "to the existence of cycles of an absolutely different nature in the animal body in which movement along a circle is not accomplished by matter but by a process, a physicochemical process which accompanies all excitation."[68] A number of examples were cited in support of the existence of circus rhythms in the complex nerve-reflex processes. Samoilov exphasized the following: "However, this far from exhausts the field of circus motions of excitation processes in our bodies. Our flow of thought not uncommonly is also of a circular nature; thus, we refer to a "circle of thoughts." To be sure, the circle here should be interpreted as a logical movement which returns us to the initial thought. To return to the initial thought does not signify mere repetition, but the introduction of something new obtained during the logical operations performed."[69]

In May 1930, at the Fourth All-Union Congress of Physiologists in Kharkov, Samoilov gave a report in which he summarized the results of the most important studies in the field of electrophysiology and outlined the tasks which had to be undertaken in the future. Samoilov himself considered making extensive use of the electrophysiological method in the study of central nervous system activity.

The works of Samoilov ("Circus Rhythm" and "Electrophysiological Method") are of particularly great importance in connection with the development of cybernetics in recent decades, the achievements of which have begun to be used extensively by biologists in their studies. Understandably cybernetics assists biologists and physiologists in the solution of some of their most important problems. At the same time, it should be mentioned that long before cybernetics expressed itself as an independent science some of the most important principles and postulates on which it was based were formulated in the studies of physiologists. In Samoilov's research, beginning with 1922, ideas are consistently developed which have the most direct bearing on the theory of regulation of the body's activity in the cybernetic sense.

V.A. Engel'gardt, G.D. Smirnov, N.A. Grigoryan,[70] I.A. Vetokhin and G.S. Yun'ev called attention to this aspect of Samoilov's activity. In 1931, in an article dedicated to the memory of A.F. Samoilov, V.A. Engel'gardt[71] wrote that Samoilov had expanded the idea of circus processes beyond the limits of the purely physical and had extended this principle to phenomena of an incomparably more complex nature, to the processes of excitation and psychosensory phenomena. G.D. Smirnov wrote that in Samoilov's statements in the article "Circus Rhythm," "it is easy to see the formulation of the 'feedback' principle which at the present time is considered an achievement of cybernetics."[72] I.A. Vetokhin wrote this about his teacher's report at the Kharkov Congress: "Aleksandr Filippovich included an exhaustive, strictly logical demonstration in his report on how deeply the innermost secrets of the body's nerve-reflex regulation can be penetrated. He showed conclusively that rhythmical activity of the central nervous system originates under the influence of striated muscle, which is not a passive executor of the commands of the central nervous system but takes an active part in the elaboration and adaption of this rhythmical activity. Therefore, an interaction is established among the various components of the motor apparatus: on the one hand, reciprocal innervation of muscles, and on the other hand the effect of the working muscles on their nerve centers. This interaction was noted by I.M. Sechenov but has been experimentally substantiated only recently, with the use of the electrophysiological method of investigation, of which A.F. Samoilov is a past master."[73] Samoilov's student, Professor G. S. Yun'ev, present director of the Department of Physiology of the Belorussian State University and editor of the collection including Vetokhin's article, writes the following in a note to Vetokhin's article: "It is fitting to add that the ideas of P.K. Anokhin formulated later in the field of the "center-periphery" problem developed the main thought advanced by A.F. Samoilov in his Kharkov report." This comment applies also to the formulations about the role of "feedback in neurophysiology"[74] frequently repeated in recent years influenced by the technical progress of cybernetics.

In connection with this, P.K. Anokhin's statements of recent years are surprising, particularly his report at the All-Union Conference on Philosophical Problems of Physiology of Higher Nervous Activity and Psychology.[75] Anokhin, repudiating the classical reflex theory in the cartesian conception as worthless in explaining the central nervous system of the intact organism, advances the concept of the functional system as the best means of surmounting the defects of this theory physiologically. Anokhin believed that not a single serious and purposeful attempt had been made in nervous system physiology to eliminate the defects of the reflex theory and "to construct a more perfect conception of central nervous activity of the intact organism."[76] Only in 1935 did he formulate the "concept of the functional system as the unit of integrative activity of the body."[77] Nevertheless, for more than a century the complex nerve-reflex activity of the body has been the subject of careful study, particularly in the Soviet Union, beginning with the early investigations of I.M. Sechenov, I.P. Pavlov and their students. V.N. Chernigovskii, E.A. Asratyan and others spoke about this at the abovementioned conference.

The study of the circus rhythm of excitation permits us to come closer to the solution of some of the most important problems of physiology. It shows primarily that self-regulatory phenomena exist in living organisms. Such a system is Sechenov's "tractile muscle sense" or Sherrington's myotatic reflex, which maintains itself until it is interrupted by certain factors. The question of the origin of the rhythm in the central nervous system is closely connected with cyclic phenomena in the body.

Sechenov confronted physiology with a number of problems which could not be solved until the present time because of the [recent] improvement of electrophysiological methods of investigation. It would be no exaggeration to state that in Sechenov's study of the "dark muscle sense," or the "tractile muscle sense" the germ of modern cybernetics is present. As a matter of fact, compare the statements of the well-known modern French neuropathologist P. Cossat[78] who wrote a book on cybernetics and the thoughts of Sechenov presented in his "Nervous System Physiology."[79] It is curious that both Cossat and Sechenov refer to the same clinical example for the purpose of confirming their ideas — the abnormal coordination of movement in persons with ataxia. Cossat writes: "In order to maintain balance in the standing position the intervention of corrective factors is constantly necessary. These factors represent a special function of an accessory circuit, which is connected with the main motor circuit and functions by means of feedback."[80]

Cossat's "corrective factors" are nothing but "sensory symbols," "the dark muscle sense" according to Sechenov. Referring to clinical observations of his friend S.P. Botkin on patients with ataxia, Sechenov wrote: "On this basis science assumes the existence of a specialized dark muscle sense, which, along with cutaneous and visual perceptions, serves, so to speak, as the most important director of consciousness in the matter of coordinating movements ... from the examples of spinal cord disease described above it evidently follows that the most important role among these directors should be ascribed to muscle sense despite its darkness, and the least important, to visual acts, despite their well-defined nature. This latter factor particularly infers that the mechanism of coordination of walking, or voluntary movement generally, through muscle sense is absolutely identical with the mechanism of coordination of reflexes of the decapitated animal in expedient movements ... The sensations from the skin and muscles accompanying the onset and all phases of muscular contraction determine the duration of each of them separately and the sequence of muscular contraction. Persons with ataxia represent proof of this again."[81] In analyzing the performance of a pianist, he wrote that for proper execution of the music, aside from everything else, the musician needs continuous muscular control. "The sensory symbols stamped in memory", Sechenov wrote, "form a number of notes in accordance with which or, more accurately, under the control of which the appropriate motor performance unfolds. Is it not precisely such notes which guide the musician when he plays a piece which he knows well in complete darkness? For every individual sound or chord is preceded by a separate position of the fingers in space with subsequent movement of them; hence, the correct execution is guaranteed by habitual sensations from the performing hand and not the hearing (or, more accurately, "not only the hearing" —N.G.). In other words, in playing in the darkness, prior to rapid movements and in parallel with them there are a number of sensory symbols which determine the successive changes in the positions of the hands."[82] It is fitting to compare Sechenov's example with what Samoilov wrote about the transmission of excitation on some circus pathways to others during the playing of a violin. Sechenov summarized the study of muscle sense in the following way: "any change in the position of the hands, legs and trunk, just as any movement of these parts, gives our consciousness, through the medium of the so-called muscle sense, silent but very definite sensory symbols that we immediately recognize the change in the position of the limb and the movement which has accomplished this change."[83]

Sechenov's study of muscle sense constitutes a major division in present-day physiology and is well known by the name of proprioception. It is unjustifiably connected with the name of Sherrington, despite the fact that in 1929 Samoilov brilliantly proved that all of the most important ideas in the study of proprioception belong to Sechenov.[84]

In Samoilov's electrophysiological studies Sechenov's study of the "dark muscle sense" has been developed further. The electrophysiological method permitted Samoilov to discover new features in already-known reflex phenomena and to understand them in greater detail. Specifically, he made a detailed study of the mechanism of the myotatic reflex, the phenomenon of muscle plasticity. It was determined that in the quadriceps femoris muscle there are two types of sensory nerve endings: stimulation of some of them leads reflexly to muscle contraction; stimulation of others, to inhibition of this reflex contraction. It was found that in the mechanism of the movements and in their innervation there is no definite fixed law by which the agonist and the antagonist divide their participation in this movement. The roles of these two muscles vary in different movements. In the light of electrophysiology the problem of the origin of central nervous system rhythm has been clarified in an absolutely new way. "Progress lies in the fact that the entire formulation of the problem has to be changed," said Samoilov. "The problem of the origin of rhythmic impulses requiring muscles for tetanus no longer depends on the properties of the nervous center wherever a dendrite rhythm is created."[85]

In this way Samoilov posed the problem of the center and the periphery. H. Mittelstedt is right when he writes: "I am convinced that today's physiologists would have had to work toward the theory of regulation themselves if it were not yet in existence."[86]

Prior to Samoilov nothing was known in Russia about electrocardiography. The status of the graphic method of study can be characterized by the words of the well-known clinician, A.Ya. Yarotskii who wrote, "The cardiac impulse alone is available for study in cardiac activity. In the study of the latter, unfortunately, most of the work has been done by instruments which do not stand up to strict criticism. The indifference of the majority of clinicians to graphic study of the cardiac impulse is explained by this."[87]

Six years after this, in Holland and ten years later in Russia the situation changed markedly. Clinical medicine received from the hands of the physiologists a fine, precise and rapid method of recording the activity of the human heart under absolutely normal conditions. Continuing the work of his first teacher I.P. Pavlov, Samoilov from the very beginning of his activity in the field of electrocardiography had brought about a "legal union between physiology and medicine" studying physiology and pathology of the human heart. By the same token, Samoilov carried out the testament of his second teacher, I.M. Sechenov, who had laid the basis for human physiology in his "Essays on Human Work Movements." Samoilov's first article on electrocardiography was published at the beginning of 1908. Shortly after this (1910), articles by the clinicians V.P. Zelenin, S.S. Steriopulo and V.L. Yanovskii and the physiologist, A.A. Yudin, were published. Similar studies were begun later in other countries. Thus, the well-known American cardiologist, P. White, became acquainted with Einthoven's string galvanometer only in 1913; Lewis in England, Winterberg and Rothberger in Vienna, and A. Hoffman in Germany began their studies after Samoilov.

In the Soviet Union electrocardiography began to be developed along two lines from the very start — experimental and clinical-physiological. Samoilov's Kazan laboratory became the center for the study of electrocardiography, and Russia became its second home.

References

[1] Waller, A.D. On the Electromotive Changes Connected with the Beat of the Mammalian Heart and of the Human Heart in Particular. — Philos. Trans. Roy. Soc. Lond., Vol. 180, p. 169. 1889.

[2] Willem Einthoven (1860—1927). — In: Collection "Nobel Prize Winners in Medicine and Physiology. 1900—1950," pp. 115—118. New York. 1953.

[3] Wenckebach, K.F. W. Einthoven. — Deutsche medizinische Wochenschrift, No. 5, p. 2176. 1927.

[4] Samoilov, A.F. Vospominaniya o prof. V. Eintkhovene (Recollections of Professor W. Einthoven). Selected articles and speeches. — Izdatel'stvo AN SSSR, p. 175. 1946.

[5] Hoogerwerf, S. Leven en werken van Willem Einthoven. Grondlegger van de Electrocardiografie. Hoo. 1955.

[6] Einthoven, W. Die galvanometrische Registrierung des menschlichen Elektrokardiogramms, zugleich eine Beurtheilung der Anwendung des Capillar-Electrometers in der Physiologie. — Pflüger's Arch. gesamte Physiol., Vol. 99, pp. 472—480. 1903.

[7] Ibid., p. 472.

[8] Einthoven, W. Le telécardiogramme. — Arch. neerland sci. exacts et natur., Ser. II, Vol. 2, p. 239. 1906.

[9] Samoilov, A.F. Vospominaniya o prof. V. Eintkhovene (Recollections of Prof. W. Einthoven), p. 155.

[10] Botkin, S.P. Izbrannye trudy (Selected Works), Vol. I.—Medgiz. 1952.

[11] Sechenov, I.M. Fiziologiya nervnoi sistemy (Physiology of the Nervous System), p. 107. St. Petersburg. 1866.

[12] Pavlov, I.P. Polnoe sobranie sochinenii (Complete Collection of Works). Vol. 6, p. 29. — Izdatel'stvo AN SSSR. 1952.

[13] Samoilov, A.F. Elektrokardiogrammy (Electrocardiograms). — Russkii Vrach, No. 33, pp. 1089—1094. 1908.

[14] Ibid., p. 1089.

[15] Ibid., p. 1092.

[16] Ibid., p. 1093.

[17] Ibid., p. 1094.

[18] Ibid.

[19] Samoilov, A.F. Organizm cheloveka kak ob"ekt fiziologicheskogo issledovaniya (The Human Body as a Subject for Physiological Investigation). Selected articles and speeches, pp. 189—190.

[20] Bykov, K.M. O klinicheskoi fiziologii (On Clinical Physiology). — Vestnik AMN SSSR, No. 1. 1948.

[21] Davydovskii, I.V. O zadachakh meditsinskoi nauki v predstoyashchem semiletii (Tasks of Medical Science in the Next Seven Years). — Vestnik AMN SSSR, No. 3, pp. 3—8. 1959.

[22] Samoilov, A.F. Elektrokardiografiya (Electrocardiography). — Arkhiv AN SSSR, folio 652, op. 1, No. 1, p. 27.

[23] Ibid., p. 1.

[24] Ibid., p. 60.

[25] Samoilov, A.F. O fotografirovanii pokazanii kapillyarnogo elektrometra (Photography of Capillary Electrometer Readings). — Fotograficheskoe Obozrenie, No. 3, pp. 1—11. 1896.

[26] Samojloff, A. Beiträge zur Elektrophysiologie des Herzens. Engelmanns. — Arch. Physiol., Suppl. Vol., p. 207. 1906.

[27] Samojloff, A. Elektrokardiogrammstudien. — Beiträge zur Physiol. und Pathol., pp. 172—188. 1908.

[28] Ibid., pp. 182—183.

[29] Ibid., pp. 186—187.

[30] Samojloff, A. Über die Vorhoferhebung des Elektrokardiogramms bei Mitralstenose. — Münch. med. Wochenschrift, No. 38, pp. 1—12. 1909.

[31] Ibid., p. 12.

[32] White, P.D. Personal Observations on the Evolution of Cardiovascular Surgery. Einthoven lecture. — University of Leyden. September 21, 1956. Leyden, 1957, p. 2.

[33] Samojloff, A. Weitere Beiträge zur Elektrophysiologie des Herzens.— Pflügers Arch. gesamte Physiol., Vol. 135, pp. 417—468. 1910.

[34] Samojloff, A. Weitere Beiträge. — Pflügers Arch. gesamte Physiol., Vol. 135, p. 417. 1910.

[35] Ibid., p. 468.

[36] Samojloff, A. Die Vagus und Muskarinwirkung auf die Stromkurve des Froschherzens. — Pflügers Arch. gesamte Physiol., Vol. 155, pp. 471—522. 1914.

[37] Dale, D. and G. Mines. The Influence of Nerve Stimulation on the Electrocardiogram. — J. Physiol., Vol. 46, p. 318. 1913.

[38] Samojloff, A. Die Vagus und Muskarinwirkung auf die Stromkurve des Froschherzens. — Pflügers Arch. gesamte Physiol., Vol. 155, p. 508. 1914.

[39] Samojloff, A. Die Aktionsströme des Froschherzventrikels bei hormonaler Vagusreizung. — Pflügers Arch. gesamte Physiol., Vol. 217, Nos. 5, 6; D. 582—597. 1927. Cited from p. 473.

[40] Samojloff, A. Die Vagus und Muskarinwirkung auf die Stromkurve des Froschherzens. — Pflügers Arch. gesamte Physiol., Vol. 155, p. 473. 1914.

[41] Samoilov, A.F. Polozhitel'noe kolebanie toka pokoya predserdiya cherepakhi pri razdrazhenii bluzhdayushchego nerva (The Positive Deflection of the Atrial Resting Current in the Turtle on Vagal Stimulation). — Izvestiya Rossiskoi Akademii Nauk, Series 6, Vol. 11, Part 2, p. 1263. 1917.

[41] Samojloff, A. Die positive Schwankund des Ruhestromes am Vorhofe des Schildkrötenherzens bei Vagusreizung (Gaskeles Phänomen). — Pflügers Arch. gesamte Physiol., Vol. 199, No. 6, pp. 479 — 594. 1923.

[42] Gaskell, W. H. Über die elektrischen Veränderungen, welche in dem ruhenden Herzmuskel die Reizung der Nervus vagus begleiten. Beiträge zur Physiologie, p. 114. 1887.

[43] Samoilov, A. Polozhitel'noe kolebanie toka pokoya predserdiya cherepakhi pri razdrazhenii bluzhdayushchego nerva (The Positive Deflection of the Atrial Resting Current in the Turtle on Vagal Stimulation). — Izvestiya Rossiskoi Akademii Nauk, Series 6, Vol. 11, Part 2, p. 1263. 1917.

[44] Einthoven, W. and A. Rademaker. Über die angebliche positive Stromschwankung und der Schildkrötenvorkammer bei Vagusreizung usw. — Pflügers Arch. gesamte Physiol., Vol. 166, p. 109. 1916.

[45] Samoilov, A. F. Polozhitel'noe kolebanie toka pokoya predserdiya cherepakhi pri razdrazhenii bluzhdayushchego nerva (The Positive Deflection of the Atrial Resting Current in the Turtle on Vagal Stimulation). — Izvestiya Rossiskoi Akademii Nauk, Series 6, Vol. 11, Part 2, p. 1265. 1917.

[46] Ibid., pp. 1275 — 1276.

[47] Arkhiv AN SSSR, file 652, op. 2, No. 211.

[48] Danilov, L. V. O vliyanii serdechnykh nervov lyagushki na tok pokoya obezdvizhennogo zheludochka (The Influence of the Cardiac Nerves of the Frog on the Resting Current of the Immobilized Ventricle). — Kazanskii Meditsinskii Zhurnal, Nos. 4 — 5, pp. 410 — 414. 1931.

[49] Orbeli, L. A. Lektsii po fiziologii nervnoi sistemy (Lectures on the Physiology of the Nervous System), 3rd edition. — Izdatel'stvo AN SSSR. p. 293. 1938.

[50] Samojloff, A. Die Stromkurve des partiel durchschnittenen Froschventrikels und die Leitung im Herzmuskel. — Pflügers Arch. gesamte Physiol., Vol. 222, No. 4, pp. 516 — 533. 1929.

[51] Zelenin, V. F. Bolezni serdechno-sosudistoi sistemy (Diseases of the Cardiovascular System). — Medgiz, p. 166. 1956.

[52] Samojloff, A. F. Die Stromkurve des partiel durchschnittenen Froschventrikels und die Leitung im Herzmuskel. — Pflügers Arch. gesamte Physiol., Vol. 222, No. 4, p. 530. 1929.

[53] Borisova, B. I. and V. S. Rusinov. O dlitel'nykh nizkovol'tnykh potentsialakh serdtsa (Protracted Low-Voltage Cardiac Potentials). — Klinicheskaya Meditsina, Nos. 7 — 8, p. 79. 1940.

[54] Samoilov, A. F. and M. N. Cheboksarov. K klinike serdechnykh blokov (s 5 kardiogrammami) (The Clinical Picture of Heart Block (with 5 Electrocardiograms)). — Kazanskii Meditsinskii Zhurnal, Vol. 22, No. 1, pp. 23 — 33. 1926.

[55] Op. cit.

[56] Samojloff, A. and A. Tschernoff. Reziproker Herzrhythmus beim Menschen. — Zs. exper. Pathol. und Therap., Vol. 71, Nos. 5, 6, pp. 767 — 777. 1930.

[57] Mines, G.R. On Dynamic Equilibrium in the Heart. — J. Physiol., Vol. 46, p. 349. 1913.

[58] Samoilov, A. F. Kol'tsevoi ritm vozbuzhdeniya (Circus Rhythm). Selected articles and speeches, p. 231.

[59] Ibid., p. 235.

[60] Ibid., p. 235.

[61] Ibid., p. 240.

[62] Ibid., p. 253.

[63] Ibid., pp. 253—254.

[64] Ibid., p. 253.

[65] Ibid., pp. 257—258.

[66] Samoilov, A.F. O perekhode vozbuzhdeniya s kletki na kletku (The Spread of Excitation from Cell to Cell). Selected articles and speeches, p. 260.

[67] Ibid., p. 225.

[68] Samoilov, A.F. Garvei i ego zaslugi (Harvey and His Contributions). Selected articles and speeches, p. 40.

[69] Ibid., p. 42.

[70] Grigoryan, N.A. Razvitie idei I.M. Sechenova v trudakh A.F. Samoilova (Development of I.M. Sechenov's Ideas in A.F. Samoilov's Works). — Voprosy Istorii Estestvoznaniya i Tekhniki, No. 4, p. 85. 1957.

[71] Engel'gardt, V.A. Anaerobnyi raspad i aerobnyi resintez pirofosfata v krasnykh krovyanykh kletkakh ptits (Anaerobic Decomposition and Aerobic Resynthesis of Pyrophosphate in Red Cells of Birds). — Kazanskii Meditsinskii Zhurnal, Nos. 4—5, pp. 496—501. 1931.

[72] Smirnov, G.D. Ritmicheskie elektricheskie yavleniya v tsentral'noi nervnoi sisteme, ikh proiskhozhdenie i funktsional'noe znachenie (Rhythmic Electrical Phenomena in the Central Nervous System, Their Origin and Functional Significance). — Uspekhi Sovremennoi Biologii, Vol. 12, No. 3, p. 325. 1956.

[73] Vetokhin, I.A. Aleksandr Filippovich Samoilov (kratkii ocherk zhizni) (Alexander Filippovich Samoilov (Brief Outline of His Life)). — In: Sbornik "Voprosy fiziologii cheloveka i zhivotnykh", No. 1, pp. 10—11, Minsk. Izdatel'stvo Belorusskogo gosudarstvennogo universiteta. 1960.

[74] Yun'ev, G.S. Primechanie k stat'e I.A. Vetokhina (Note on I.A. Vetokhin's Article). — In: Sbornik "Voprosy fiziologii cheloveka i zhivotnykh," No. 1, p. 11.

[75] Anokhin, P.K. Metodologicheskii analiz uzlovykh problem uslovnogo refleksa (Methodological Analysis of the Key Problems in the Study of the Conditioned Reflex. Proceedings of the Conference on Philosophical Problems of Physiology of Higher Nervous Activity and Psychology), 78 p., Moskva. 1962. (Manuscript.)

[76] Ibid., pp. 46—47.

[77] Ibid., p. 70.

[78] Cossat, P. Cybernetics. Translated from the French under the editorship of and with a foreword by P.K. Anokhin. — IL. 1958.

[79] Sechenov, I.M. Fiziologiya nervnoi sistemy (Physiology of the Nervous System). St. Petersburg. 1866.

[80] Cossat, P. Cybernetics, p. 47.

[81] Sechenov, I.M. Fiziologiya nervnoi sistemy (Physiology of the Nervous System), pp. 243—244—245.

[82] Sechenov, I.M. Uchastie nervnoi sistemy v rabochikh dvizheniyakh cheloveka (1900). (Participation of the Nervous System in Human Work Movements (1900)). Collection of Works, Vol. 2, pp. 458—459. Moskva. 1908.

[83] Ibid., p. 458.

[84] Samoilov, A.F. I.M. Sechenov i ego mysli o roli myshtsy v nashem poznanii prirody (I.M. Sechenov and His Ideas on the Role of Muscle in Our Knowledge of Nature). Selected articles and speeches, pp. 43—69.

[85] Samoilov, A.F. Elektrofiziologicheskii metod v uchenii o refleksakh (Electrophysiological Method of Studying Reflexes). Selected articles and speeches, p. 306.

[86] Mittelshtedt, H. Regulation in Biology. — In: Sbornik "Protsessy regulirovaniya v biologii," Translated from the German under the editorship of and with a foreword by P.K. Anokhin. IL. 1960.

[87] Yarotskii, A.Ya. Kardiogramma, snyataya neposredstvenno s serdtsa (Electrocardiogram Taken Directly from the Heart). — Bol'nichnaya Gazeta Botkina, pp. 13—14. 1897.

E.M. Senchenkova

V.N. LYUBIMENKO'S STUDIES OF CHLOROPHYLL AND THEIR MODERN DEVELOPMENT

> "The interaction between light and the green leaf gives rise to one of the most remarkable processes on earth. The essential role played by the substance chlorophyll found in the green leaf is the subject now to be considered." (K.A. Timiryazev. Sobranie sochinenii (Collected Works), Vol. 1, pp. 343—344, Moskva. 1937.

1. SCIENTIFIC ACTIVITIES OF V.N. LYUBIMENKO AND HIS STUDIES ON PHOTOSYNTHESIS

September 1962 was the twenty-fifth anniversary of the death of the famous Russian plant physiologist, Vladimir Nikolaevich Lyubimenko (1873 - 1937). The entire scientific activity of this outstanding scientist was dedicated to understanding the plant kingdom and the processes taking place in it. Nature always irresistibly attracted his curiosity.

Lyubimenko's biological education began at the Agricultural School in Kharkov (1886 —1892), which he entered at the age of 13 in accordance with his parents' wish that he acquire a profession as soon as possible. He graduated as an agronomist, and was admitted to the Petersburg Institute of Silviculture in 1894. The lectures of the outstanding Russian botanist I.P. Borodin stimulated Lyubimenko's interest in botanical investigation. Since the Institute did not confer diplomas permitting independent research in botany, he became, in 1900, an extramural student at the Physicomathematics Faculty of the Petersburg University and graduated with honors in 1902. This marked the beginning of his scientific career. In 1903—1904 he visited the Strasburger's renowned cytological laboratory in Bonn and then the laboratory of the French plant physiologist G.Bonnier, after which he chose plant physiology as his main subject, concentrating on photosynthesis and related problems. In 1905 the Department of Forestry granted him permission to proceed with his scientific activities in France, in the laboratories of Bonnier at the Paris University, the Sorbonne and Fontainebleau. Here he continued the investigations on photophily and shade tolerance of forest trees which he had begun in 1904 during his first visit. The results were published in the monograph, "Influence of Light of Various Intensities on the Accumulation of Dry Matter in Photophilous and Shade-Tolerant Plants" (Vliyanie sveta razlichnoi napryazhennosti na nakoplenie sukhogo veshchestva u svetolyubivykh i tenevynoslivykh rastenii), 1909.

Lyubimenko returned from France determined to continue his investigations on the effect of light on plants. He therefore readily agreed to establish a laboratory of plant physiology on the sunny coast of south Crimea on behalf of the Nikitskii Botanical Garden. In the face of great difficulties Vladimir Nikolaevich enthusiastically persevered in the project and finally succeeded in establishing the laboratory and assembling a small staff of physiologists. He was the first plant physiologist to undertake research in the Nikitskii Botanical Garden.

From 1908 to 1912 Lyubimenko, in collaboration with N.A. Monteverde, investigated the formation of chlorophyll, its precursor, protochlorophyll, and the yellow pigments accompanying it, rhodoxanthin and lycopene. He quantitatively determined the accumulation of chlorophyll, xanthophyll and carotene by spectrocolorimetry. For these investigations the two authors received the Akhmatov Academy Award in 1913.

During the Crimean period of his scientific career Lyubimenko studied the concentration of green pigment and formation of chlorophyll in plants (1908), the effect of light of various intensities on chlorophyll synthesis in higher plants (1909), the growth of fruits and seeds (1910) and budding in trees (1910). He also investigated the assimilation and production of dry matter on exposure to white and colored light (1910), lycopene and its relationship to chlorophyll (1914), leaf nutrition and treatment of tree diseases (1914), etc. At the same time he undertook the important task of determining the content of chlorophyll in plants of various geographical latitudes. In 1910 he defended a thesis at the Petersburg University, on "The Chlorophyll Content of Chloroplasts and Photosynthetic Energy" (Soderzhanie khlorofilla v khlorofillovom zerne i energiya fotosinteza). In the same year this monograph was published in the "Trudy Peterburgskogo obshchestva estestvoispytatelei" (Transactions of the Petersburg Society for Natural Research). In a contest held by the Academy of Sciences in 1912, Lyubimenko was awarded the Buitenzorg Fellowship for his investigations. This fellowship, alternately awarded to a botanist and to a zoologist, provided for exploration in tropical countries and entailed work in the laboratory of the well-known Buitenzorg Tropical Botanical Garden in Java. Within the short period of 8 months Lyubimenko managed to travel through Australia and the Malay Archipelago and conduct physiological investigations in the Buitenzorg Garden.

Lyubimenko was interested in the quantitative determination of chlorophyll in leaves of tropical plants. The great abundance of plant species in the Buitenzorg Garden enabled him to determine the chlorophyll content of more than 400 tropical species. In addition, he examined the yellow and red pigments of fruit chromoplasts. His mastery of quantitative and qualitative analytical methods for the study of pigments, which he had developed in conjunction with Monteverde, enabled Lyubimenko to cover this vast field in only two months.

Shortly after his return from the Tropics Lyubimenko left the Nikitskii Botanical Garden, and in January 1914 he was given charge of the small physiological laboratory of the Peter the First Botanical Garden in Petersburg (now known as the Botanical Institute of the Academy of Sciences of the USSR). Later he became head of the Plant-Physiology Department, and finally, during his last years, head of the Department of Experimental Botany.

This last period was the most fertile. In March 1917 he obtained his Ph. D. at the Petersburg University for the famous monograph "The Transformation of Pigments in Plastids of Intact Plant Tissues" (O prevrashcheniyakh pigmentov plastid v zhivoi tkani rasteniya), 1916. This was the first thesis defended in Petersburg after the fall of the Czarist government.

For this work Lyubimenko received the Akhmatov Award conferred by the Academy of Sciences in 1918.

The October Revolution opened up vast possibilities for investigation by so enthusiastic a scientist as Lyubimenko.

Although the principal object of Lyubimenko's investigations remained the role of leaf pigments in photosynthesis, during this period his work covered a wide range of subjects. His studies of photosynthetic pigments included those of algae as well as of higher plants. His investigations of plastids were designed to clarify the state of chlorophyll in intact leaves and its connection with the chloroplast proteins. He was also interested in chlorophyll synthesis by plants and investigated the following aspects of photosynthesis: adaptation of plants to light; functional energy of the leaf in the process of photosynthesis; and the effect of protoplasm excitation on photosynthesis. He was especially interested in photoperiodic adaptation.

It would be difficult to review here all Lyubimenko's publications during this extremely productive period (totaling about 200). Some investigations were not completed and remained unpublished. The following appeared as separate publications: "Life of the Plant. A Short Botanical Manual for Farmers" (Rastenie i ego zhizhn'. Kratkoe rukovodstvo po botanike dlya sel'skogo khozyaina), 1921; "Pigmentation of Plants" (Okraska rastenii), 1924 (in collaboration with V. A. Brillian); "Matter and the Plants" (Materiya i rasteniya), 1924; "Weeds of Our Fields" (Sornye rasteniya nashikh polei), 1925 (in collaboration with M.M.Il'in); "Early Spring Plants" (Rannie vesennie rasteniya), 1926 (in collaboration with E.V. Vul'f); "Fall Plants" (Osennie rasteniya), 1928 (in collaboration with E.V. Vul'f); "Photosynthesis and Chemosynthesis in the Plant Kingdom" (Fotosintez i khemosintez v rastitel'nom mire), 1935; "Plant Control" (Upravlenie rasteniem), 1936; etc.

All Lyubimenko's works, from his first study "Chlorophyll Formation in Darkness" (Ob obrazovanii khlorofilla v temnote), 1905 to his extensive monograph, "Photosynthesis and Chemosynthesis in the Plant Kingdom" (1935), testify to his comprehensive analysis of the investigated phenomena and his close attention to the numerous related factors of internal and external environment.

E.R.Gyubbenet, who worked under Lyubimenko for many years on the study of plant pigments, wrote: "V.N. Lyubimenko's methods of investigation were derived from a broad biological outlook. He regarded the plant and its vital activities in relation to one another; he did not separate them or isolate the plant from its environment. His study of plastid pigments, mainly chlorophyll, was closely connected with the studies of photosynthetic activities.[1]"*

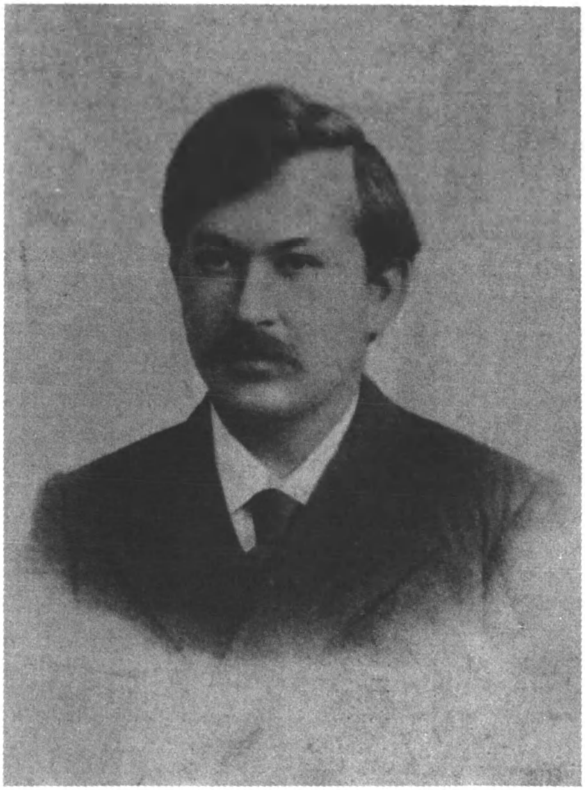
Lyubimenko analyzed the results of his investigations both as an experimental physiologist and as a biologist and Darwinist. He was interested not only in the factors inducing phase phenomena but also in how they came about through natural evolution, i.e., the origin of chlorophyll, the evolution of photosynthesis, etc. The knowledge acquired by Lyubimenko in his investigations was usually applied to problems of general biology, such as the physiological principles of inheritance of the pigment plastids, the genetic relationship between chlorophyll and hemoglobin, etc.

Lyubimenko was rewarded for his achievements in botanical and physiological research, as well as for his administrative and pedagogical work, and his efforts to popularize science, by being elected Corresponding Member of the Academy of Sciences of the USSR (1922) and Member of the Academy of Sciences of the UkrSSR. The versatility of this gifted physiologist won him fame both in Russia and abroad.

---

* [For this and other footnotes appearing in this text, see "Notes" at the end of this paper.]

This industrious scientist left a vast scientific legacy of over 270 works (some of them still unpublished), encompassing the most diverse problems in cytology, taxonomy, biochemistry and plant physiology. His extensive creative activity included applied botany, the practical utilization of scientific data in agriculture, extensive administrative and pedagogical work, and popularization of science.



V.N. Lyubimenko (1873—1937)

This review will deal only with Lyubimenko's investigations in his main field: photosynthetic apparatus, green plastids and their chlorophyll content. His works on this subject represent significant milestones in the development of plant physiology, both in Russia and abroad.

In the last quarter of the 18th century it was already known that only green plants (their green parts) can absorb carbon dioxide from the air and release oxygen. However, it took some time to prove that the green color of plants is due to the presence of microscopic structures in their cytoplasm, namely chloroplasts (A. Comparetti, 1791)[2], the green color being induced by the pigment chlorophyll (Pelletier and Caventou)[3].

After the 1850's the chloroplasts (previously called chlorophyll granules), and the chlorophyll dissolved in them, were thoroughly investigated. Kayser found that over 600 publications had been printed on chlorophyll by the beginning of the 20th century[4]. Toward the 1920's there were two main trends in the study of chromoplast pigment: 1) chemical — aiming at discovery of the chemical composition and structure of chlorophyll; 2) biological — aiming at discovery of the physiological and biological role of this pigment.

Among the famous chemists and biologists such as Sachs, Timiryazev, Pringsheim, Sorby, Borodin, Tsvet, Willstätter, Lyubimenko, etc., who investigated chlorophyll, Russian scientists have been prominent in research on the chemistry of chlorophyll from the biological aspect, as well as on the composition and structure of this pigment as related to its physiological function in photosynthesis. This approach to chlorophyll and its role in the life of the plant was first formulated by K.A. Timiryazev and subsequently adopted and developed by many other scientists including Vladimir Nikolaevich Lyubimenko.

2. CHLOROPHYLL — AN INTRICATE PROTEIN COMPLEX

Lyubimenko started to investigate the composition and structure of chlorophyll in the 1920's and, on the basis of certain chemical and optical properties of this phytochrome, he arrived at definite conclusions on the state of chlorophyll in the plastids.

In the 19th century Timiryazev[5] and Reinke[6] had already suspected that the chlorophyll of intact chloroplasts was in some way linked to protein. Pringsheim[7] assumed that the green pigment was linked with the fatty substances of the plant cell. He dedicated a separate work to hypochlorin, a hypothetical colorless "oily carrier" of chlorophyll. However, the state of chlorophyll in the intact plant remained hypothetical. In 1897 the famous Russian biochemist M.V. Nentskii wrote: ".... As to the structure of chlorophyll and the substance to which it is linked in the plant cell, we are still in the dark."[8]

The first experiments in this direction were undertaken by M.S. Tsvet at the end of the 19th century. As soon as the first stages of his investigations were completed, he wrote: "I assume that the above-mentioned pigment (chlorophyll or chlorophyllan, according to Tsvet's terminology — E.S.) is only a group of atoms split off from a more complex, unknown substance which should be designated chlorophyll."[9] In another article on the same subject he suggests that "chlorophyll (or better, chlorophyllan) represents a product and forms a part of the protein molecule."[10] This hypothetical chlorophyll-protein compound he designated chloroglobin (by analogy with hemoglobin).[11]

Tsvet's hypotheses on the presence of chloroglobin in plants, although published both in Russia and abroad,[12] were not fully appreciated. Opposition came from such well-known investigators of chlorophyll as Monteverde[13] and the Czech chemist Czapek.[14] Tsvet did not abandon his hypothesis, and maintained: "In order to fully investigate chloroglobin what must first be determined is the nature of hypochlorin (the protein component of chloroglobin — E.S.); next, the nature of the green and yellow principles; and finally, if possible, the nature of the chemical bond apparently linking these components."[15] After several preliminary communications Tsvet published the monograph "Physicochemical Structure of Chlorophyll Granules" (Fizikokhimicheskoe stroenie khlorofill'nogo zerna)[16], in which physicochemical properties of chloroglobin and methods of isolating this substance are described in a special section. Subsequently, Tsvet's efforts were chiefly directed toward the development of a chromatographic method of analysis.[17] These finally resulted in the isolation, for the first time, of pure chemical preparations of chlorophyll *a* and *b*.

Lyubimenko investigated the state of chlorophyll in plants from all possible aspects. He opposed Willstätter's assumption[18] that the chlorophyll is found in the plastids in a simple colloidal dispersion and pointed out that the preparations obtained by Willstätter did not correspond to pigments of intact plastids. On the basis of sound experimental findings, Lyubimenko suggested (1921) the existence of a close link, possibly a chemical bond, between chlorophyll and protein stroma of the chloroplasts.[19] In 1923 he published a detailed description of his methods as well as a brief critical review on the investigations of others on the nature of chlorophyll in the chloroplasts.[20]

In his repeated analyses of aqueous leaf extracts of various plants, Lyubimenko invariably detected the stable protein-chlorophyll character of these solutions. His findings were substantiated by qualitative analyses with protein precipitating reagents, i.e., tannin, alcohol, acetone, etc. Natural chlorophyll lost its stability upon treatment with acids and protein-coagulating substances. Chlorophyll solutions lost their transparency on boiling, their green color became deeper, but no precipitation was observed. "Thus," Lyubimenko writes, "the link between pigment and protein in plastids is strong, and a chemical bond inevitably suggests itself. It will seem quite possible that the chlorophyll of intact plastids is a pigmented protein compound if we recall that hemoglobin (which is similar to chlorophyll) is also chemically linked with the blood proteins."[21] Lyubimenko designated this hypothetical chlorophyll-protein complex, natural chlorophyll, as distinguished from chlorophyll in a molecular solution.

Natural chlorophyll is an intricate protein complex consisting of chlorophyll *a* and *b*. and carotenoids (carotene and xanthophyll). Its maximum absorption bands lie in the 675—680 mμ range, like those of intact leaves. The pigment solution is transparent in low concentrations, becoming opalescent with increased concentration. D.I. Sapozhnikov (1948)[22] established that on exposure to light natural chlorophyll reduces silver nitrate, and its green protein is precipitated by methanol, ethanol, acetone or ammonium sulfate. Alcohol concentrations above 50% dissolve the precipitate leaving the protein colorless.

Lyubimenko emphasized in particular the protective role of the pigment-protein bond. Indeed, the resistance of natural chlorophyll on exposure to light, acids and oxygen resembles that of the green pigment of leaves. Its resistance, however, is sharply reduced by boiling or freezing, or when treated with protein-denaturants. Then, the maximum absorption bands shift to the 660—670 mμ range which is characteristic of chlorophyll solutions in organic solvents.

L. A. Nezgovorov[23] revealed in the 1950's that cytoplasmic proteins as well as their degradation products may also protect the chlorophyll. He observed that addition of peptone to isolated chloroplasts prevented chlorophyll disintegration. He assumes, however, that proteins do not enhance the stability of chlorophyll outside the complex but only prevent denaturation of the plastids as a whole. It seems that these two cell components (protein and chlorophyll) reciprocally protect each other. Zirkle[24] observed that proteins are more easily broken down by proteolytic enzymes in etiolated leaves than in green leaves.

D. I. Sapozhnikov[25], while agreeing that proteins enhance the pigment's stability, suggests that the presence of chlorophyll in a complex of high-molecular compounds affects the energetic state of the pigment molecule, a fact of utmost importance for the detection of its ability to participate in photosynthesis. This assumption was confirmed by A. A. Krasnovskii and G. P. Brin[26] who demonstrated that chlorophyll linked by various bases to the molecule's center exhibits increased affinity to the electron, i. e., enhances oxidizing ability. In their opinion this phenomenon explains the protein's activating effect on the photochemical properties of chlorophyll in nature.

Having proved the existence of a complex bond between the pigment and the proteins of the plastids in nature, Lyubimenko advanced the hypothesis that, by analogy, the chlorophyllogen constitutes a complex of protein and protochlorophyll. Accordingly, chlorophyll synthesis can take place only from protochlorophyll combined with protein, i. e., from chlorophyllogen. Protochlorophyll cannot be converted into chlorophyll when separated from the protein. The protein component accounts for the high sensitivity of chlorophyllogen to external factors. Chlorophyllogen conversion into chlorophyll may take place in dead tissue provided that the structure of the protein complex is intact. All protein denaturants (of any intensity) destroy chlorophyllogen: the pigment component of the latter is released as protochlorophyll which, in its free state, cannot be converted into chlorophyll.

Thus, Lyubimenko suggested that in the biosynthesis of chlorophyll there is an additional stage in which an intermediate pigment is formed. The intermediate pigment is not protochlorophyll but a more complex compound of this pigment with protein, namely, chlorophyllogen. On this principle, in collaboration with N. N. Gortikova[27] he visualized the following sequence in the process:

$$\begin{array}{lcl} \text{leucophyll} & \rightarrow & \text{chlorophyllogen} \\ \text{(protein +} & & \text{(protein + proto-} \\ \text{chromogen)} & & \text{chlorophyll)} \end{array} \quad \left\{ \begin{array}{l} + \text{ enzyme} = \text{chlorophyll (protein + chlorophyllins)} \\ + \text{ light} = \text{chlorophyll (protein + chlorophyllins)} \\ + \text{ protein denaturants} = \\ = \text{protochlorophyll + protein denaturants} \end{array} \right.$$

According to this scheme the transformation of chlorophyllogen is the final stage of a process in which one complex pigmented protein compound is transformed into another. This bold assumption on the chemistry of the natural-chlorophyll molecule and the complex pigmented protein evoked no comment during the first years after its publication, either in Russia or abroad. In 1927 Noack concluded, on the basis of his investigations, that "the state of chlorophyll in intact leaves can best be described as a pigment adsorption of the chloroplast protein in the form of a monomolecular layer."[28]. Noack pointed out that experimental substantiation of Lyubimenko's hypothesis on the nature of the chlorophyll—protein bond would seem imperative.

Numerous investigations in this direction proceeded during the 1930's: Mestre (1930), Stoll (1936), Mommarts (1938), Smith (1938), Price and Vikov (1938), Stoll and Wiedemann (1938 and 1941), Katz and Wassink (1939), and French (1938).[29] They revealed that chlorophyll-protein compounds occur not only in the chloroplasts of higher plants but also in those of algae and bacteria. French[30] and Krasnovskii et al[31] established that in aqueous extracts of purple bacteria the pigments occur in an intricate complex with proteins, similar to that detected in natural chlorophyll. The absorption maxima of these extracts corresponded to the same wavelengths (800, 850, and 890 mμ) as in intact bacteria. The famous German chemist Stoll,[32] who ignored Lyubimenko's hypothesis for 15 years, mentioned it first in 1936 and later (in an article by Stoll and Wiedemann, 1938), wrote of "confirming" and "adding to" observations of this Russian physiologist, on aqueous extracts of "chloroplastin" from the leaves of various plants. Subsequently, references to Lyubimenko's works frequently occurred in other foreign publications. In 1941 Anson, in an article entitled "On Lubimenko's Extracts of Chlorophyll-Protein,"[33] praises the interesting and promising observations of this Russian scientist and voices his dismay at the lack of response at the time of publication.

Later numerous investigations proved that the complex compounds of prosthetic groups with their protein carriers are encountered in all plant pigments. Rabinowitch,[34] Sapozhnikov, et al[35] and Nishimura and Takamitsu[36] found that carotenoids are linked to proteins. Analogical bonds with protein carriers were revealed in such little-known pigments as chlorophyll *c* and *d*, bacteriochlorophyll, bacteriopurpurin, phycoerythrin, phycocyanin, chlorofucin, various carotenes and carotenoids[37].

Studies on the nature of enzymes greatly clarified the state of chlorophyll in the plastids. Willstätter found that the enzymes are "symplectic", consisting of two components. Kraut developed this hypothesis and demonstrated that the symplectic enzymatic system can be described as a complex comprising an active group (agon) and a colloidal carrier (feron).[38] Stoll[39] described the chlorophyll of the chloroplasts as a dual system, similar to that of the enzymes and especially of hemoglobin, consisting of a prosthetic group and a colloidal (protein) carrier. He designated it chloroplastin omitting to mention that the term was coined by M. S. Tsvet (1901). The "symplectic" notion — a two-component system of chlorophyll in the plastids — was further developed by Anson,[40] Gyubbenet[41] and Osipov.[42]

The concept that chlorophyll is a pigmented protein or enzyme steadily penetrated the scientific literature in the 1940's. Smith (1938) wrote: "It seems that the classic investigations on the chemistry of chlorophyll dealt only with the prosthetic group of the extremely complicated and specific catalyst. This agent is apparently analogical to hemoglobin and to the catalase, cytochrome, and yellow respiratory types of enzymes." This opinion, as well as subsequent investigations, prove that Lyubimenko was right in designating chlorophyll a "green protein".

Lyubimenko designated the chlorophyll-protein complex natural chlorophyll. Other authors coined various terms for the same group, e.g., phyllochlorin (Mestre, 1930), chloroplastin (Stoll, 1936) and even photosynthin (French, 1940), in order to adapt the term not only to plants with chloroplasts but also to algae and bacteria. Rabinowitch (1951) suggested the term chloroglobin or chromoglobin for the chlorophyll-protein complex. He thus ignored the earlier introduction of the term by Tsvet (1898).

This controversy on the designation of a compound long since described and named can be ascribed to the ignorance of most foreign scientists of the investigations of Tsvet and Lyubimenko — the first to study the chlorophyll-protein bond in intact leaves. Although the achievements of these two Russian scientists were acclaimed by foreign scientists in the 1930's-1940's, Tsvet is not even mentioned by Rabinowitch when treating the problem in his three-volume monograph on photosynthesis, while Lyubimenko's natural chlorophyll is described, strangely enough, as a hypothetical complex.

The existence of this complex is now firmly established. The Soviet physiologists T. N. Godnev and O. P. Osipova[44] have almost ascertained the nature of the chemical forces linking the chlorophyll to the protein molecule. They suggest that this link emerges from the acid groups of the protein molecules and the residual valences of the nitrogen atoms in the pyrrole rings of the chlorophyll molecules. This bond is labile, varying according to plant species and age. E. Smith,[45] D. I. Sapozhnikov and T. G. Maslova[46] demonstrated that Mg is not essential in the chlorophyll-protein bond since, when the Mg splits off, the remaining group (pheophytin) does not lose its bond with the protein.

The stoichiometric relationship between chlorophyll and protein is not as stable as that existing in hemoglobin or cytochrome. Data supplied by various scientists disagree. According to A. Neish[47] chlorophyll is linked to protein in the granules at the same ratio as hemoglobin: one chlorophyll molecule to one Svedberg unit (protein with molecular weight 1700). According to Smith there are three molecules of chlorophyll a and one molecule of chlorophyll *b* to each Svedberg unit. According to Rabinowitch[48] the ratio of chlorophyll to protein in the chloroplasts is at least four times as great as that of hemoglobin or cytochrome. A. A. Krasnovskii[49] points out that no strict, constant ratio of chlorophyll to protein had hitherto been found in pigment-protein complexes. He showed that this ratio may range between three to ten chlorophyll molecules to each protein unit. Godnev and Osipova assume that the varying ratio of chlorophyll to protein in the chloroplasts and granules, as well as the labile bonds, may indicate that chlorophyll does not form so stable and definite a complex as does hemoglobin, for instance.

The successful investigations of chlorophyll-protein complexes led to the experimental production of such complexes from chlorophyll preparations and protein compounds. The first steps in this direction were taken by Eisler and Portheim[50] who used horse serum as a protein carrier for chlorophyll. The spectrum of green proteins thus obtained resembled the spectra of leaves. These proteins were water soluble, fluoresced and were able to photo-oxidize. Noack[51] adsorbed chlorophyll on various substances, viz., proteins (albumin, globulin, legumin, casein), peptones, kaolin, and aluminum hydroxide. These adsorbed chlorophyll complexes, fluoresced and proved to be more stable on exposure to light and oxygen than the molecular chlorophyll in organic solvents.

After a long interval, investigations in this direction were resumed by Osipova,[52] Sapozhnikov,[53] Lepeschkin,[54] and Rodrigo[55]. The following were used as protein components for the experimental production of chlorophyll-protein complexes – conglutin, zein, gliadin and other fat-free plant proteins (Osipova), albumin and lecitin (Lepeschkin), and protein from white-geranium leaves (Rodrigo). Sapozhnikov prepared complexes with lipoprotein of both animal (yolk granules of chicken eggs) and vegetal origin (pulp of carrot roots, beet, turnip, etc.). The optical properties (absorption spectrum), fluorescence and photochemical activity of the artificial complexes obtained resembled those of natural chlorophyll.

Although Lyubimenko's assumption that natural chlorophyll exists in the form of a pigment-protein complex is now widely accepted, the state in which this complex occurs in leaves is still unknown. Noack[56] assumes that most of the chloroplasts' pigment is linked to the protein of the stroma (in a colloidal state). The rest of the pigment is in a lipid-soluble state. This opinion is shared by Seybold and Egle[57], whereas Rabinowitch[58] does not regard this dual theory as scientifically well founded. In his opinion, chlorophyll is bound in the plastids with both proteins and lipids. This was confirmed experimentally by B. Sing and N. Anantha[59]. In various plants, however, various states of the chlorophyll and its bonds with proteins and lipids were revealed.

Godnev et al.[60] attempted chlorophyll extraction with nonpolar solvents, viz., benzene, petroleum ether and hexane. The results obtained suggest that chlorophyll may occur in leaves in two states: dissolved in lipids, or coupled with proteins and lipids. In the first, chlorophyll is extracted by petroleum ether; in the latter, it can be extracted only by polar solvents such as alcohol and acetone. These authors regard the chlorophyll-protein-lipid complex as an enzymatic system. Osipova[61] advanced the view that chlorophyll occurs in the plastids both in a lipid solution and in a complex with lipoprotein. She attempted to relate the state of the chlorophyll to that of the other components in the complex, i. e., proteins and lipids.

Following the study of chlorophyll phosphorescence of green algae Brugger and Frank[62] suggest that one part of the chlorophyll is probably linked only to proteins; the other to both proteins and lipids. After study of the fluorescence and photochemical activity of chlorophyll preparations, Krasnovskii and Brin[63] concluded that these phenomena are induced solely by that part of the pigment occurring in a state of molecular dispersion. Later, Krasnovskii[64] suggested the probability that chlorophyll occurs in leaves in three states: a) bound to lipoproteins; b) in lipid solution; c) in reserve form (buffered), resembling a coagulated colloid phase. The latter is photochemically inactive.

Subsequently, Krasnovskii et al.[65] developed the theory that there are two forms of chlorophyll in plants: a) monomeric, in the form of a chlorophyll-protein-lipid complex with an absorption maximum of 670 mμ (chlorophyll 670); b) aggregated (chlorophyll-protein-lipid) · n with an absorption maximum of about 678 mμ (chlorophyll 678). The same authors showed that in etiolated plants reverting to their green color the first chlorophyll form appears first, whereas the second form appears several hours later; this was inferred by the shift of the absorption maximum from 670 to 678 mμ. The optical properties and the character of photochemical activity of these forms testify that the monomeric chlorophyll 670 participates in photochemical reactions and causes fluorescence, whereas the aggregated chlorophyll 678 is photochemically inactive, does not fluoresce, and thus represents the reserve form. Nevertheless, the light energy absorbed by the aggregated form of chlorophyll can be transferred to the monomeric form.

The above-mentioned authors attribute the variations detected in the ratio of monomeric to aggregated forms to the plant species, age and physiological condition, as well as to the influence of environmental factors. A state of equilibrium exists among these chlorophyll forms in the chloroplasts:

$$C \cdot PL \rightleftarrows C + PL \rightleftarrows (C + PL)\,n \rightleftarrows C + (PL)n,$$

where C = chlorophyll, P = proteins, L = lipids, n = the state of aggregation.

In an attempt to separate the monomeric from the aggregated chlorophyll by ultracentrifugation, Krasnovskii and co-workers obtained the aggregated form in the sediment whereas they found that the monomeric form enriched the supernatant. Their findings were established by spectroscopic analysis.

Spectroscopic methods were used by Godnev and co-workers[66] in analysis of the chlorophyll-protein bond in green leaves of various plants grown in different media and subjected to increasingly higher temperatures (within the range of 20° to 80°C). These experiments established that changes in the absorption spectra lie within the range of temperatures causing protein denaturation. If the main bulk of chlorophyll in the leaves is bound to proteins, the disintegration of the bond at higher temperatures between these two components is due to protein denaturation. Fully green leaves require a higher temperature rise in order to show a shift in the absorption maxima. This may be explained by the prevalence of monomeric forms in the leaves when they first acquire their green color and the later appearance of aggregated chlorophyll, when bonds have been established between the molecules.

A. Shlyk and V. Prudnikova[67] observed that pigment extraction with a non-polar solvent (petroleum ether) at first proceeds quite rapidly then abruptly slows down. The authors suggest that this fall in the rate of extraction is due to the presence of two forms of chlorophyll in the leaves and that the extraction of one form leads to the abrupt arrest of the process. Comparing their results with those obtained by Krasnovskii in experiments with extracted leaf suspensions, Shlyk and Prudnikova noted its quantitative correspondence to the monomeric fraction. In their opinion the chlorophyll fraction extractable with petroleum ether corresponds to the lipid-soluble chlorophyll found in equilibrium with the monomer of the protein-lipid chlorophyll.

Smith et al.[68] and Shibata[69] observed that during the formation of chlorophyll *a* from protochlorophyll, some forms of chlorophyll *a* are transformed into optically different ones. Smith assumes that the aggregated state of the chlorophyll does not account for the shift of the maximum-absorption spectrum from 670 to 678—680 mμ, thus disagreeing with Krasnovskii and Rabinowitch[70] who attribute the spectral shift, from 670 to 678 mμ, to the transformation of the "disorganized" chlorophyll-protein complex into "organized" monomolecular layers, and maintain that all chlorophylls exhibit photochemical activity and fluorescence.

An interesting approach to the nature of chlorophyll 670 is advanced by Godnev and co-workers[71]. According to their investigations this monomeric form is a chlorophyll-protein complex in which, on further illumination, phytol and then lipids take part. In their experiments, the reaction of chlorophyll with protamin (a protein with well-pronounced basic properties) results in a substance with a spectrum similar to that of chlorophyll 670.

The state of natural chlorophyll and its transformations are now being studied both in Russia and abroad directly in the live cell, by means of fluorescent spectrophotometry. This has enabled many scientists to establish the existence of fluorescent and nonfluorescent forms of chlorophyll with greater accuracy. Differential spectroscopy, the most recent method of spectroscopic analysis, is now successfully employed.

Soviet physiologists made a series of investigations on the changes occurring in the chlorophyll-protein complex during the growth of individual plants. M.M.Okuntsov[72] and Godnev and Osipova[73] demonstrated that more chlorophyll can be extracted from young leaves than from old ones. They conclude that the stability of the chlorophyll-protein bond is enhanced with age. Osipova and Timofeeva[74] assume that the change in the state of chlorophyll is related to the physicochemical changes of the protein component of the complex. The most prominent changes connected with the aging of leaves are the increased size of the protein molecules and the greater viscosity of the protein. According to Maslova,[75] aging of plants is accompanied by diminishing stability of the chlorophyll-lipoprotein bond. Her investigations on the state of chlorophyll in leaves of various ages show that the stability of the chlorophyll-lipoprotein component increases from the beginning of leafing reaching its maximum, in evergreen plants, after the first year.

Godnev and Osipova[76] observed that the chlorophyll-lipoprotein complex is more stable in spermatophytes than in sporophytes. Maslova[77] found that the bond of the chlorophyll-lipoprotein complex is more stable in modern gymnosperms and angiosperms than in the primitive species.

All these findings confirm beyond doubt Lyubimenko's conclusions on the state of chlorophyll in the live plant cell. It now seems certain that the chlorophyll fraction, extractable by organic solvents, investigated at the end of the 19th and the beginning of the 20th century, represents the prosthetic group of a more intricate complex. E. R. Gyubbenet correctly stated: "Timiryazev divided the history of investigation of the chemistry of chlorophyll into two periods — before Willstätter and after him. It is no less true that Lyubimenko's discovery, which completely upset our representation of the chlorophyll molecule, marks the beginning of a third period (from 1921 on) during which the true nature of the chlorophyll molecule was established."[78]

3. NATURE OF CHLOROPHYLLOGEN AND PROTOCHLOROPHYLL IN PLANTS AND THE PREREQUISITES OF THEIR FORMATION

The problem of chlorophyll biosynthesis has occupied many scientists since the middle of the 18th century. The hypothesis advanced by Price on the formation of a colorless substance which he designated "chlorophyll-chromogen", acting as a precursor of chlorophyll in the vegetal cell, was widely adopted in scientific circles. Though variously designated "colorless chlorophyll" (Schleiden, 1855), "chlorophore" (Böhm, 1859), "leucophyll" (Sachs, 1862), and "etiolin" (Pringsheim, 1874 and Wiesner, 1877), all authors agreed that chlorophyll is not directly synthesized from its initial components, but from a colorless precursor. Timiryazev not only established the presence of a chlorophyll precursor, which he designated protophyllin, but also attempted artificial production of this substance by reduction of chlorophyll (1885 and 1866)[79], and then tried to isolate it from plants (1889)[80]. Timiryazev studied the photochemical function of protophyllin as a precursor of chlorophyll, paying special attention to its light absorption within the orange range of the spectrum.

Timiryazev's investigations were continued by the Russian physiologist N. A. Monteverde[81] at the Biological Laboratory of the Petersburg Botanical Garden. In alcohol extracts of etiolated seedlings of various plants Monteverde revealed a peculiar green pigment which he designated protochlorophyll. The absorption spectrum of this pigment fell within the orange sector of the spectrum. In his opinion, protochlorophyll is formed in the dark, accumulating in the plastids of etiolated plants in such small amounts that its green coloration is masked by the yellow pigments. Protochlorophyll is converted into chlorophyll even at the slightest illumination. According to Timiryazev[82], Monteverde's protochlorophyll is identical with his protophyllin.

From then on the term "protochlorophyll" was generally adopted for the chlorophyll precursor. Numerous investigations on this substance were subsequently undertaken, both in Russia (Lyubimenko, Buslov, Godnev, Sapozhnikov, Krasnovskii, et al., etc.) and abroad (Noack, Scharfnagel, Seybold, Fischer, Beck, Rabinowitch, etc.).

Stimulated by the work of Timiryazev and Monteverde, Lyubimenko carried out several spectroscopic investigations of alcoholic extracts of green-pigmented seeds. The results were summarized and published in 1906[83] "This work", he wrote later, "enabled me to prove that the green substance coloring the inner membrane of squash seeds is not chlorophyll but a peculiar pigment whose absorption spectrum is similar to that of protochlorophyll revealed by N. A. Monteverde in etiolated plants. Later, I learned that N. A. Monteverde had preceded me in the study of green membranes of squash seeds and had obtained similar results, although he did not manage to publish them. In order to avoid such a coincidence in the future N. A. Monteverde and I decided to continue the investigations on pigments of Cucurbitaceae together."[84]

Lyubimenko and Monteverde worked in conjunction for four years (1909—1912) in the Nikitskii Botanical Garden and their cooperation was extremely fruitful. During that period they investigated the seeds of numerous cucurbitaceans whose rich protochlorophyll content provided them with the material required. They could thus study all the properties of protochlorophyll and were able to identify it with the protochlorophyll of etiolated seedlings.

During 1909 the investigations were mainly focused on the optical properties of protochlorophyll and chlorophyll and the possibility of converting the first into the second. It was found that alcoholic extracts of both pigments are almost indistinguishable to the naked eye and emit an identical red fluorescence. Comparison of their absorption spectra (Figure 1) revealed that the spectrum of protochlorophyll lacks the first absorption band, which is characteristic of chlorophyll, and that its second absorption band lies to the left of the corresponding chlorophyll band. The remaining three bands of both spectra appear at approximately the same wavelengths. More accurate reports were published by Lyubimenko and Monteverde on the absorption spectra of protochlorophyll from alcoholic extracts of luffa-seeds (Figure 2). The following bands clearly appear in an average concentration: II (λ640—620 mμ), III (λ590—570 mμ), IV(λ540 — 525 mμ); bank VI (λ450 — 430mμ) appears in weak solutions. Both chlorophyll and protochlorophyll produce a brown derivative when treated with acids and a green derivative when treated with caustic potash.

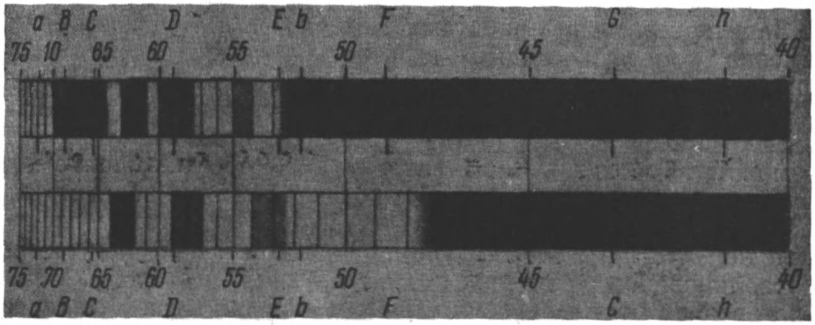


FIGURE 1. Absorption spectra: upper — intact green leaves; lower — alcoholic solution (average concentration) of luffa protochlorophyll (after N. A. Monteverde and V. N. Lyubimenko, 1911)

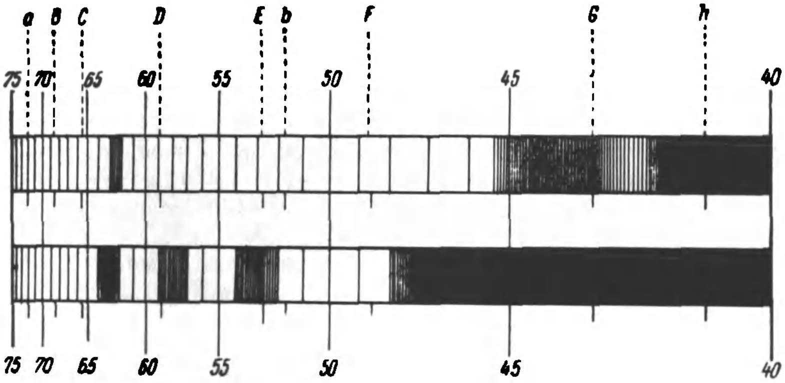


FIGURE 2. Absorption spectrum of an alcoholic solution of luffa protochlorophyll: upper — low concentration; lower — average concentration (after N. A. Monteverde and V. N. Lyubimenko, 1909)

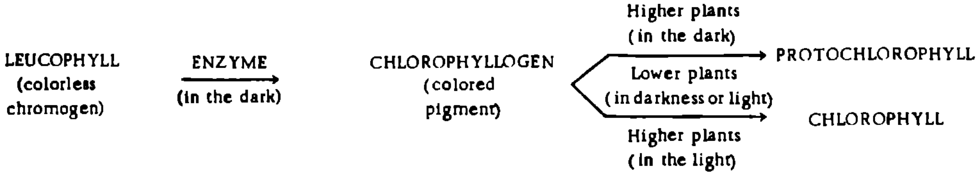
Initially, Monteverde assumed that conversion of protochlorophyll into chlorophyll followed immediately upon illumination of etiolated leaves. Since there was no doubt that the protochlorophyll of etiolated plants was identical with that found in squash seeds, it was decided to ascertain whether the protochlorophyll of intact seed membranes is converted into chlorophyll when exposed to light. It was found that "protochlorophyll formed in the plastids of seed membranes is not converted into chlorophyll under the influence of light."[85] These authors assumed that the appearance of chlorophyll in the plastids of green plants is not preceded by the formation of protochlorophyll but of a peculiar intermediate pigment which they designated chlorophyllogen.

This theory of Monteverde and Lyubimenko was not readily accepted by their contemporaries. Liro[86] suggested that chlorophyll synthesis is a purely photochemical process in which a colorless substance (leucophyll) turns into a green pigment under the direct influence of light without intermediate stages. Isachenko[87] also shared this point of view. Liro and Isachenko did not deny the existence of protochlorophyll but assumed that normal, intact plastids of etiolated plants contain only a colorless substance (leucophyll), which turns into chlorophyll under the influence of light. Should the normal state of the cell be disturbed by chemical or physical agents, this chromogen would be either completely destroyed or turned into a peculiar green pigment, protochlorophyll.

This difference of opinion, between Liro and Isachenko on the one hand and the above-mentioned authors on the other, on the nature of protochlorophyll and chlorophyll synthesis prompted Lyubimenko and Monteverde to publish further data to strengthen their theory (1911)[88]. They did not succeed in isolating chlorophyllogen from plants because of its great lability which leads to its conversion into a more stable substance, i.e., protochlorophyll, under the influence of solvents. They reported the spectroscopic analyses of chlorophyllogen in intact etiolated seedlings of wheat and luffa and its conversion into chlorophyll. As soon as etiolated seedlings are exposed to light, the first two absorption bands of chlorophyllogen shift from the λ 700—680 mμ and λ650—630 mμ ranges to the λ 680—660 mμ and λ 640—620 mμ ranges. Band I becomes stronger and band II weaker. No other absorption bands were detected for chlorophyllogen in addition to the two mentioned because this pigment is very rapidly converted into chlorophyll during the photochemical reaction. This results in the appearance of absorption bands III and IV, which are characteristic of chlorophyll. Experiments with monochromatic light showed that the conversion of chlorophyllogen into chlorophyll is most rapid under red and orange illumination. The second, weaker absorption bank lies within the blue orange (λ480-405 m μ). Pure green light has very little effect.

These and a series of other observations and experiments with green plant pigments finally proved that the hypothesis, advanced by Lyubimenko and Monteverde was correct. In their summary we read that: "... Intact plastids of green plants may accumulate two different green pigments: chlorophyll and protochlorophyll. Both are rather stable, although the latter is more so. The two pigments emerge from a third substance which we designate chlorophyllogen. Chlorophyllogen, unlike its derivatives chlorophyll and protochlorophyll, is a very labile substance. Under the influence of light, it is very rapidly converted into chlorophyll, or, if treated with different chemical agents, into protochlorophyll. The conversion of chlorophyllogen into chlorophyll can also take place in the dark by means of some unknown substances formed by plants which are able to grow green in darkness."[89] The same was observed by A. Schmidt[90].

The results of thorough investigations on the synthesis of chlorophyll and its precursors were summarized in one of the chapters of Lyubimenko's Ph. D. thesis, "On Transformations of Pigments in the Plastids of Live Plant Tissues" (O prevrashcheniyakh pigmentov plastid v zhivoi tkani rastenii), 1916. Pigment transformation in plastids has been schematically represented by Lyubimenko and Monteverde, as follows:



Lyubimenko was convinced that the green color of plants is acquired under the influence of temperature. He assumed that temperature is involved not in the photochemical transformation of chlorophyllogen into chlorophyll but in the dark reactions preceding chlorophyllogen formation. His own investigations and Greilach's observations[91] led Lyubimenko to assert that chlorophyllogen is transformed into chlorophyll at a threshold light intensity. The reaction was not observed at light intensities below this threshold. In order to facilitate his work, Lyubimenko used green light which was, in his opinion, weak and least effective.

E. R. Gyubbenet,[92] Lyubimenko's co-worker, established in 1928 that chlorophyllogen and protochlorophyll synthesis do not take place at a low temperature. Traces of chlorophyllogen could be detected only 24 hours after the beginning of an experiment at 6°C. A rise in temperature led to an increased amount of pigment. The rate of chlorophyllogen disappearance was much higher. Only traces of this pigment remained after a 30 second exposure to light, and conversion into chlorophyll was complete after two minutes. No quantitative relation could be established between the rate of chlorophyllogen disappearance and of chlorophyll accumulation. The explanation offered by Gyubbenet suggested that chlorophyllogen was not the only source of chlorophyll synthesis, and also that chlorophyllogen might appear and be converted into chlorophyll immediately upon illumination.

N. N. Gortikova and D. I. Sapozhnikov[93] demonstrated that chlorophyllogen synthesis is inhibited by dehydration and completely ceases upon treatment with a 10% potassium chloride solution. Since chlorophyllogen synthesis is an enzymatic process including an oxidation-reduction reaction, it became evident that it is strongly affected by the rH.

In time, Lyubimenko changed his opinion on the nature of chlorophyllogen. He suggested that natural chlorophyll is a complex of the green pigment and the protein of chloroplasts. He also regarded chlorophyllogen as an intricate complex of protochlorophyll and a protein carrier. It follows that the relationship between chlorophyllogen and protochlorophyll is analogous to that existing between natural chlorophyll of the plastids and chlorophyll extracted from them by various solvents. The chlorophyllogen complex consisting of a pigment and a protein is also destroyed when treated with solvents. Protochlorophyll resulting from this treatment is then equivalent to the denatured phytochrome of etiolated seedlings.

Various opinions are now held on chlorophyllogen. T. N. Godneva writes: "It is still difficult to decide whether chlorophyllogen, i. e., protochlorophyll linked to a protein stroma, should be considered the precursor of chlorophyll, as postulated by Lyubimenko, or whether chlorophyllogen slightly differs from protochlorophyll, as is evident even from the chemical structure. (This alternative appears more probable to us)."[94] In the extensive foreign literature on chlorophyll synthesis the term chlorophyllogen is not used unless referring to Lyubimenko's works, which are well known abroad. The American scientist E. Rabinowitch thinks that "... the existence of "leucophyll" and "chlorophyllogen" of the Lyubimenko-Monteverde scheme was not quite substantiated experimentally."[95] On the other hand, E. R. Gyubbenet inclines to the opinion that "the green pigment of etiolated seedlings is the precursor of chlorophyll, i. e., chlorophyllogen. As to protochlorophyll, which is extractable from etiolated seedlings by organic solvents and exhibits an optically stable pigment, it can hardly be regarded as an intermediate product of chlorophyll synthesis. It is rather a product of chlorophyllogen disintegration."[96] Gyubbenet points out that most scientists make the gross mistake of including the quite different pigment chlorophyllogen in the term "protochlorophyll", although she concedes that the great instability of chlorophyllogen when exposed to light and solvents accounts for the difficulty of differentiating between the two in experimental work, and consequently recognizes that scientists almost invariably deal with either protochlorophyll or chlorophyll.

Research on the chemical composition of chlorophyll advanced during the 1930—1940's thanks to the important work of M. S. Tsvet[97], R. Willstätter and A. Stoll[98] on the chemistry of chlorophyll and its derivatives. Noack and Kiessling[99] revealed the chemical kinship between protochlorophyll and chlorophyll reflected by the presence of magnesium and alcoholic groups (phytol and methyl) in the molecules of both pigments. Their data show that protochlorophyll is distinguished from chlorophyll by the lower oxygen content, and that chlorophyll is formed through oxidation of protochlorophyll.

In 1937, using Tsvet's method of adsorption analysis, Seybold[100] obtained from squash seeds two chromatographic fractions which he interpreted as protochlorophyll *a* and *b*. He thus substantiated his hypothesis[101] that protochlorophyll *a* and *b* are converted by light into chlorophyll *a* and *b*, respectively. Subsequent investigations by A. A. Krasnovskii and K. K. Voinovskii[102] did not confirm the existence of two forms of protochlorophyll. T. N. Godnev[103] assumes that chlorophyll *b* emerges from a transformation of the chlorophyll *a* molecule.

Valuable data on the chemical structure of protochlorophyll were brought to light by G. Fischer. In 1939, Fischer and co-workers succeeded in synthesizing protochlorophyll by incorporating phytol and magnesium in 2-vinylpheoporphyrin A_5.[104] The synthetic substance obtained proved to be identical with the natural pigment. Fischer demonstrated that protochlorophyll is a complex magnesium compound of phytol ester 2-vinylpheoporphyrin A_5. A semi-isolated double bond, 7—8, is found in its molecule in the fourth pyrrole ring. This bond is hydrogenated in the chlorophyll molecule (Figure 3). Consequently, the chemical composition and molecular structure of these pigments permit us to consider the protochlorophyll as a product of chlorophyll *a* oxidation with the elimination of two hydrogen atoms in positions 7 and 8. The spectrum of protochlorophyll is also similar to that of chlorophyll *a*, with an absorption maximum at 635 mμ.

Fischer's observations on the stability of protochlorophyll can be explained, to a certain extent, by the more recent results obtained by Krasnovskii et al.[105] who found that protochlorophyll in living cells (as well as chlorophyll, see p. 163, et seq.) occurs in two forms, viz., an active form (protochlorophyll 635) and an inactive one (protochlorophyll 645). The authors define the active forms as forms whose photobiochemical transformations can be determined experimentally, such as transformations of protochlorophyll into chlorophyll or reversible transformations of chlorophyll during photosynthesis. Another criterion for activity is the photooxidation of atmospheric oxygen by the pigment. Krasnovskii and Kosobutskaya observed that illumination of etiolated leaves or of their colloidal solutions containing protochlorophyll with an absorption maximum in the red range (635 mμ) led to the appearance of an active form of chlorophyll with an absorption maximum at 670 mμ (chlorophyll 670). Intense illumination leads to the destruction of both protochlorophyll and the freshly formed chlorophyll. Different results were obtained in similar experiments with protochlorophyll of seed membranes.

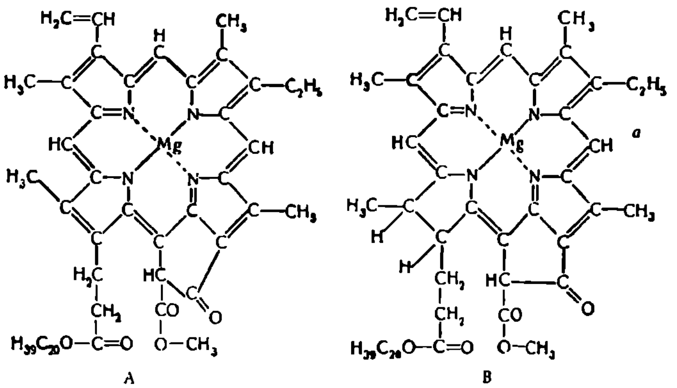


FIGURE 3. Structural formula of protochlorophyll (A) and chlorophyll *a* (B) (from H. Fischer "Fortschritte der Chlorophyllchemie", 1940)

Illumination of squash seeds, seed membranes, and their colloidal solutions did not induce the synthesis of chlorophyll. The above authors observed that the maximum absorption bands of seed protochlorophyll corresponds to $\lambda = 645\,m\mu$, i.e., by $10\,m\mu$ fukher in the red range of the spectrum than etiolated leaves. They assume that the "inactivity" of this form of protochlorophyll is principally determined by the aggregation which protects the pigments bound to proteins and lipids against irreversible photochemical disintegration.

These findings helped to clarify many observations made in connection with protochlorophyll investigation. The conclusions drawn by Timiryazev and Monteverde on the transformation of protochlorophyll of etiolated seedlings, into chlorophyll, do not contradict the later views of Monteverde, Lyubimenko and Fischer on the stability of this pigment obtained from seed membranes of Cucurbitaceae. Both views are correct, since the ratio of the active to the inactive states of the pigment changes in the growth process of the plant. In the course of seed germination the inactive form of protochlorophyll is converted into an active form, the reverse process taking place during seed formation. The ratio of the active to the inactive states of the pigment is also influenced by the physiological state of the plant and environmental factors.

Rothemund,[106] clearly demonstrated that protochlorophyll is not identical in all plants by revealing the different absorption spectra of this pigment in corn, hemp, etc. Qualitative spectral differences in the protochlorophyll of different plants were also established by E. D. Buslova.[107] In the etiolated germinating seeds of 62 species (from 17 families) Buslova found considerable spectral differences in the precursor of chlorophyll (as described by Timiryazev and Monteverde). The phytochrome, designated by the author "primary protochlorophyll", exhibited strikingly different spectra from those of chlorophyll. Primary protochlorophyll, intermediate protochlorophyll and — upon illumination — chlorophyll occasionally appeared in succession in the same etiolated seeds during germination e. g., in sunflower, corn, rhubarb, common tobacco, etc. Buslova reported an even more complex protochlorophyll in germinating lilac seeds. However, Godnev[108] maintains that the "primary protochlorophyll" described by Buslova is in fact protopheophetin, a substance chemically similar to protochlorophyll but without magnesium.

At any rate, the existence of protochlorophyll in plants is now well established. Protochlorophyll is nowadays regarded either as a direct precursor of chlorophyll synthesis in the plant or as a more stable form of the precursor of chlorophyll.

4. PATTERNS OF CHLOROPHYLL BIOSYNTHESIS AND THE ROLE OF PROTOCHLOROPHYLL IN THIS PROCESS

Although Timiryazev, Monteverde and Lyubimenko were the first to investigate protochlorophyll and suggest possible procedures of chlorophyll biosynthesis and the role of protochlorophyll in the process, their studies did not provide an adequate chemical interpretation. The first attempt at such an interpretation was undertaken by the Soviet physiologist T. N. Godnev (1926).[109]

Fischer's observations confirmed Lyubimenko's opinion that protochlorophyll represents an oxidized form of chlorophyll arising as a by-product during synthesis. Lyubimenko assumed that the first stage of chlorophyll synthesis is oxidation of the chlorophyll precursor, i.e., the hypothetical leucophyll. This hypothesis was first forwarded by Preisser (1884), later developed by Lyubimenko in collaboration with Monteverde[110], and elaborated in Lyubimenko's dissertation.[111]

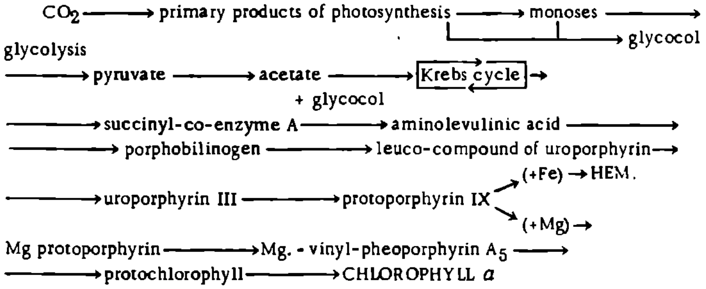
On the other hand Scharfnagel,[112] Noack and Kiessling,[113] etc., suggested that chlorophyll is synthesized directly from protochlorophyll with the participation of light. According to their analytical data protochlorophyll is chemically very similar to chlorophyll, and characterized by a high reduction rate. This led the above-mentioned authors to the conclusion that protochlorophyll is the direct precursor of chlorophyll in the process of chlorophyll synthesis. Their findings were confirmed by Frank's[114] observations on the "action spectrum" of the green color emergence which he found to be very close to the absorption spectrum of protochlorophyll.

Protochlorophyll could not be definitely considered the precursor of chlorophyll while scientists were producing highly contradictory data. Fischer emphasized that protochlorophyll is stable and cannot be reduced by light; whereas Krasnovskii and Kosobutskaya[115] noted that the protochlorophyll of ground, etiolated germinating seeds is converted into chlorophyll in a few minutes. Godnev suggested that in plants the synthesis of pyrrole rings, as raw materials, precedes formation of the porphyrin skeleton of chlorophyll. He supported the existence of such a stage in chlorophyll synthesis with a series of data. Godnev (1928—1959), Eiler (1929), Granik (1948—1954), Cookson and Rimington (1954), Shemen (1956), and Shlyk (1956)[116] developed more elaborate schemes of the pyrrole synthesis in the preliminary stage of biosynthesis and subsequent formation of the porphyrin skeletal structure of chlorophyll. These authors utilized the data available on hemesynthesis, especially of the initial stages, since the initial stages of chlorophyll, and hemoglobin synthesis, are identical up to the formation of protoporphyrin. The inclusion of a Mg atom into the protoporphyrin molecule leads, through a series of transformations, to chlorophyll synthesis, whereas the inclusion of iron results in heme formation. The extensive use of stable and radioactive isotopes, and of various chromatographic methods in the biological (especially biochemical) investigations greatly advanced study of this problem.

The principal stages of initial chlorophyll biosynthesis are the condensation of pyrrole compounds with free alpha-positions, resulting in the formation of a more complex precursor of chlorophyll, i.e., a colorless leuco-compound corresponding to leucophyll. Oxidation of leucophyll leads to the formation of protochlorophyll. According to Rabinowitch[117] it is still unknown whether this pigment is an intermediate product in chlorophyll synthesis or a by-product, as suggested by Lyubimenko. An increasing number of observations speak in favor of the former assumption. It is difficult to accept the alternative suggested by Rabinowitch that the presence of protochlorophyll traces in fully green barley plants (as observed by Koski and Smith, 1948) is either a by-product or an indispensable precursor in chlorophyll synthesis. Any by-product should accumulate in the organism, and this cannot be said of protochlorophyll.

Should protochlorophyll be considered the precursor of chlorophyll, as most scientists assume, it would follow that the final stage of chlorophyll synthesis is a reduction, and not an oxidation, reaction, whereby chlorophyll is formed by the reduction of the leuco-compound at the double bond of the pyrrole ring. Godnev assumes that "it is not improbable that such an initial oxidation followed by reduction takes place in plastids of the living cells."[118]

Up to now, the most complete scheme of chlorophyll biosynthesis is that proposed by the Soviet biochemists Godnev[119] and Shlyk[120] who studied the process with the aid of labeled elements:


CO₂ → primary products of photosynthesis → monoses →
glycocol
glycolysis
→ pyruvate → acetate → Krebs cycle →
+ glycocol
→ succinyl-co-enzyme A → aminolevulinic acid →
→ porphobilinogen → leuco-compound of uroporphyrin →
(+Fe) → HEM.
→ uroporphyrin III → protoporphyrin IX
(+Mg) →
Mg protoporphyrin → Mg. - vinyl-pheoporphyrin A5 →
→ protochlorophyll → CHLOROPHYLL a

According to Godnev and co-workers,[121] conversion of protochlorophyll into chlorophyll takes place in two stages. In the first, protochlorophyll is hydrogenated at the double bond 7—8 into chlorophyllid A; subsequently, the chlorophyllid is esterified by phytol. Occasionally, protochlorophyll contains phytol, when conversion is effected in one stage. Rubin[122] maintains that chlorophyll synthesis is connected with oxidation processes catalyzed by Fe-proteins (enzymes). Thus, the solid ground of experimental research has recently replaced earlier theoretical speculations in the studies of chlorophyll synthesis in plastids of living cells.

5. INFLUENCE OF EXTERNAL FACTORS ON SYNTHESIS AND ACCUMULATION OF CHLOROPHYLL

Lyubimenko investigated chlorophyll synthesis in various conditions of light, temperature, oxygen supply, etc.

Influence of light on chlorophyll synthesis. The most important factor in this process is light. Lower plants can synthesize green pigment in the dark, whereas most plants, i.e., all the higher plants, synthesize chlorophyll only in the presence of light. Lower plants which normally become green in the dark accumulate still greater amounts of chlorophyll in light.[123] The significant role of light in chlorophyll synthesis was well known by the sixties of the 19th century, when Sachs[124] and Famintzin[125] proved experimentally the dependence of pigment formation on light intensity. They established that green color is acquired more slowly on direct exposure to sunrays than on weaker illumination.

Wiesner (1877)[126] established the minimum light intensity required for chlorophyll synthesis according to the appearance of fluorescence in an alcoholic extract of etiolated germinating seeds. He found that different plants acquire the green pigmentation at the same minimum light intensity. Greilach[127] observed that chlorophyll synthesis ceased in etiolated sprouts of Lepidium sativum and Phaseolus vulgaris at a light intensity of 1/8 candlepower held at a distance of 5 m. Chlorophyll synthesis could be induced either by enhanced light intensity or greater proximity of the light source.

Lyubimenko's[128] experiments on synthesis of chlorophyll in oat and barley sprouts confirmed the existence of such an illumination minimum. Lyubimenko defined it as the lower threshold of light. He aimed at establishing the exact quantitative relationship between light intensity and chlorophyll accumulation in live tissue. He considered the experimental solution of this problem important for the solution of the general problem of light influence on chlorophyll synthesis.

Lyubimenko placed a 10-candlepower electric bulb in a dark box with an opening (diameter 1 cm) covered with a piece of ground glass. This light source was placed at a distance of 1 m from germinating barley seeds. At 50 cm from the diaphragm, the light intensity would decrease proportionately to the square of the distance. Lyubimenko determined the amount of chlorophyll in the seeds by a spectrocolorimetric apparatus built by himself. Results obtained after 10 hours of exposure are summarized in Table 1.

TABLE 1

Distance from light source (in cm)	Light intensity (%)	Amount of chlorophyll (%)
400	6.25	0
300	11.10	21
200	25.00	45
100	100.00	100

These data show that a certain threshold of light intensity has to be passed in order to induce chlorophyll synthesis. This threshold marks the initial stage of chlorophyllogen conversion into chlorophyll. Light intensity above this threshold leads to a proportionate increment of the chlorophyll output up to a point at which further increase of light intensity suppresses chlorophyll synthesis. Lyubimenko suggested that the accumulated chlorophyll retained part of the light falling on the leaf, impairing the illumination conditions required by chlorophyllogen conversion into chlorophyll. He assumed that light may also induce a reverse transformation of chlorophyll into a colorless substance (possibly even leucophyll) and that the rate of this process increases with the increase of light intensity.

These experiments enabled Lyubimenko to draw some general, preliminary conclusions on the influence of light on various stages of chlorophyll synthesis. In the summary of his work we read the following: "In the first stage chlorophyllogen formation proceeds either in the absence of light or at a very poor illumination; in the next stage chlorophyllogen is converted into chlorophyll under the effect of stronger illumination. This stage apparently represents a typical photochemical reaction. In the third stage another photochemical conversion of the green pigments leads to the quantitative decrease of chlorophyll. A comprehensive understanding of the entire process can evidently be achieved only through detailed investigation of each one of these stages."[129]

Thus, in addition to the quantitative determination of the lower threshold of light intensity in chlorophyll synthesis, Lyubimenko also developed his earlier hypothesis on the existence of an upper light threshold. In 1907—1909 he established the optimum light intensity for chlorophyll synthesis in light-grown seedlings.[130] He revealed that in many plants the amount of chlorophyll accumulated on exposure to full daylight is lower than on exposure to diffuse light as obtained by exposure to daylight screened with white paper. The optimum light intensity (corresponding to maximum chlorophyll accumulation) fluctuates widely for various plant species: for photophilous plants it approximates full daylight, whereas for sciophytes it is considerably below full daylight.

In subsequent investigations Monteverde and Lyubimenko[131] found that the light optimum for chlorophyll synthesis in common plants may lie below the intensity of diffuse daylight. The following results were obtained with pea seedlings grown in diffuse daylight and in light intensities lowered by 1, 2, 4, or 6 paper layers (Table 2).

TABLE 2

Light intensity	Chlorophyll accumulation (%)		Chlorophyll increment after 10 days (%)
	after 14 days	after 24 days	
Full daylight (diffuse)	9.2	46.1	501
Lowered by one paper layer	19.4	53.1	273
" " two paper layers	17.5	40.1	217
" " four paper layers	10.9	23.3	213
" " six paper layers.	9.7	15.2	156

It is evident from the tabulated data that the greatest accumulation of chlorophyll proceeded in diffuse daylight lowered by one layer of clean paper.

As to the increment of chlorophyll during ten days, the authors found "evident signs of adaptation" in sprouts grown in full daylight. The chlorophyll content of this group increased by a factor of five, whereas the increment in the shaded sprouts was significantly lower. Lyubimenko's co-worker E. R. Gyubbenet,[132] recorded the same gradual "adaptation" to stronger light intensities in 9-day-old etiolated wheat seedlings. She found that during the first 8 hours of exposure the light provided by a 50 w lamp proved the most favorable, but with the gradual adaptation of the plant to light, the intensity of the light source could be increased to 300w. The rate of chlorophyll synthesis upon illumination with 1500w was already equal to that of a 300 w illumination after 24 hours. Gyubbenet concluded that light not only induces the photochemical synthesis of chlorophyll but also accelerates and catalyzes reactions providing the raw material for it.

Lyubimenko[133] also determined the optimum light for tree leaves at different sites on the crown of the tree. Thus he revealed that more chlorophyll is contained in shaded leaves of linden (Tilia parvibolia) and in slightly shaded leaves of maple (Acer platanoides) and alder (Allus incana).

These and other factors led Lyubimenko to conclude that "for each tree species there is a specific optimum light intensity at which the maximum amount of chlorophyll is accumulated in its leaves. Any deviation from this optimum necessarily leads to a lower concentration of the pigment." [134]

The influence of light on the synthesis and accumulation of chlorophyll was studied both quantitatively and qualitatively. A wide range of publications have appeared on the effect of particular sectors of the spectrum in this process. However, the subject remains controversial. The recorded discrepancies might be explained by the various methods (not always accurate), employed by the authors, of obtaining monochromatic light and of quantitative determination of chlorophyll and other objects of investigation. Two conflicting views were held in the 1850's — 1860's. Timiryazev assumed that photosynthesis is most intensive within the red zone of the spectrum, whereas the German physiologists Sachs[135] and Wiesner[136] held that it takes place within the yellow sector. Timiryazev's works stimulated further experiments. Monteverde and Lyubimenko[137] and Schmidt[138] clearly established that red light is optimal for chlorophyll synthesis. In support of Timiryazev, Schmidt pointed out that "chlorophyll synthesis takes place only within the range of light absorbable by chlorophyll". Lyubimenko[139] found that optimal chlorophyll synthesis occurs within the red zone, which is absorbable by chlorophyllogen, as well as within the blue zone. He did not succeed in determining the absorption of blue rays because of the rapid transformation of chlorophyllogen into chlorophyll upon illumination. Lyubimenko assumed that this absorption should exist by analogy with subsequently, Seyer (1928), Rudolf (1933), Simonis (1938), Vent (1939), Rubin and Chernavina (1955), etc., confirmed that red and red-orange light is optimal for chlorophyll synthesis in etiolated seedlings of various plants. The Soviet physiologist A. B. Brandt[140] demonstrated that pigment accumulation in plants is influenced by both spectral composition and light intensity. At low light intensities pigment accumulation is more rapid in the red range of the spectrum. This may be due to the maximum accumulation of carbohydrates in this zone of the spectrum, as well as to the presence of better conditions for the synthesis of the chlorophyll precursors. At high light intensities, more pigments accumulate in the blue range because strong light changes the rate of the oxidation-reduction processes in plants.

Chlorophyll biosynthesis in the dark. Careful investigations conducted at the beginning of the 20th century (Artari[141], Burgerstein[142], Bittner[143], etc.) revealed that in lower plants (algae, mosses and lichens) chlorophyll synthesis also occurs in darkness. J. Sachs[144] observed the same for coniferous plants. The shortcomings of these observations are that they were based on visual estimation of the comparative chlorophyll content accumulated in the light or dark. No reliable methods were available for the accurate determination of chlorophyll content, and minute quantities of pigment synthesized in the dark could not be estimated.

Chlorophyll biosynthesis in the dark was studied by Lyubimenko at the beginning of his scientific career. A brief communication on chlorophyll synthesis in coniferous plants[145] appeared in 1905.

For determination of the qualitative and quantitative differences between chlorophyll synthesized in the dark and in light, Lyubimenko selected seedlings of the conifers Pinus pinea, Pinus silvestris and Picea excelsa.

The solution of both problems required the use of spectroscopy. The advantages offered by this highly sensitive method are obvious. Thus, the minute amounts of chlorophyll synthesized by larch seedlings in darkness would not have been detectable by any other method. Furthermore, since, at the beginning of the 20th century, the chemical composition of chlorophyll had not been definitely established, spectroscopy proved to be the only reliable method for the comparative determinations of pigments and their derivatives extracted from plants. The first problem, the possible qualitative difference between dark and light synthesis, was easily solved.

Comparative spectroscopic analyses revealed no qualitative difference. The second task — the quantitative determination of chlorophyll — proved more difficult. As an indicator of varying concentrations in the extracts Lyubimenko recorded the shifts occurring in the first absorption band of chlorophyll, between Frauenhofer lines B and C (680—640 mμ), and revealed that the width and intensity of the first band of the chlorophyll spectrum increased proportionately to the concentration of the pigment. Lyubimenko prepared five standard solutions of chlorophyll extracts, in decreasing concentrations of 1:2:3:4:5, and determined the variations of width in their first absorption band. The experimental alcoholic extracts of chlorophyll from seedlings grown in light and in the dark were then compared with those of the standard solutions. The comparison revealed a significant drop of chlorophyll synthesis in the absence of light. For pines, for instance, the chlorophyll output in darkness attained only one-quarter of that recorded in light. Bulgakova and Engel'[146] confirmed the accuracy of these results in subsequent determinations, carried out with more precise and modern equipment.

Lyubimenko observed that the chlorophyll concentration in etiolated seedlings varies with their age. Thus, the amount of chlorophyll per unit of live mass synthesized by 16-day-old etiolated seedlings of Italian stone-pine was only one-tenth of that produced by 8-day-old seedlings grown under the same conditions. Because of the inaccuracy of the obtained data the author could not take into account the changes of intensity in the absorption band of the chlorophyll. Already in this first study of chlorophyll Lyubimenko paid attention to other physiological processes of the plant, i.e., respiration and assimilation.

In order to clarify the effect of respiration on chlorophyll synthesis in the dark, and the role of the organic reserve compounds in this process, Lyubimenko undertook similar investigations of the spring branches of spruce. He found that from buds opening in the dark only etiolated shoots, in which no chlorophyll could be detected by spectroscopic analysis, are produced. These results confirm Sachs's observations. According to Lyubimenko, the reason is quite obvious: "Upon transference of the branches to darkness assimilation ceases. The winter reserves suffice only for the respiration of old leaves and for the energy required by the respiration and growth of the young shoots. Chlorophyll synthesis, which is not indispensable for the preservation of life, ceases for lack of material. In seedlings chlorophyll is also synthesized in darkness, though in comparatively small amounts, owing to the ampler supply of reserve substances".[147]

The rate of CO_2 assimilation, measured during half an hour, revealed that assimilation is more intense in coniferous seedlings grown in light than in those grown in the dark (see Table 3).

TABLE 3

Species	Amount of CO_2 (in % per unit of live mass of the seedlings)			
	grown in the dark		grown in the light	
	11 day old	20 day old	11 day old	20 day old
Pinus pinea	0.80	4.46	1.84	6.89
Pinus silvestris	1.87	—	4.45	—
Picea excelsa.	1.61	1.29	2.47	3.06

These were the first experimental data indicating the direct connection between the intensity of photosynthesis and the amount of chlorophyll in the plant.

Lyubimenko termed his communication on chlorophyll synthesis in the dark "preliminary". Subsequently, his interest encompassed the factors affecting chlorophyll synthesis in light and in dark, as well as the influence of environmental factors on this process.

In the course of his study of general biological problems Lyubimenko investigated specific minute details. His co-workers Bulgakova and Engel' established that in spruce chlorophyll is readily synthesized in darkness, whereas in pine this is accomplished with great difficulty and larch almost completely loses this capacity. The larch thus represents a transitional stage from coniferous to leafy plants. The phylogenetic position of the conifers is intermediate between pteridophytes and angiosperms, being more closely related to the former. This conclusion wholly substantiates N. I. Kuznetsov's[148] hypothesis on the close relationship of the three largest groups of higher plants — following the phylogenetic sequence pteridophytes — gymnosperms — angiosperms — and the closer kinship between the first two.

Lyubimenko[149] assumed that chlorophyll synthesis in the dark depends upon the intensity of oxidation taking place in the chloroplasts in the presence of free oxygen as well as upon the effect of these — most probably oxidizing — enzymes catalyzing this process.

This was subsequently investigated by Schmidt.[150] He observed that chlorophyll synthesis in the dark takes place in coniferous seedlings, provided that the endosperm is normal. When the endosperm was experimentally removed from the embryo no chlorophyll was synthesized, even though all nutritional requirements were satisfied and ground endosperm was added to the medium. On the other hand the seedlings rapidly turned green if supplemented with a small fraction of intact endosperm, irrespective of whether it originated from the same coniferous species or from a different one. Lyubimenko agreed with Schmidt's hypothesis that coniferous endosperm contains an enzyme which catalyzes chlorophyll synthesis in the dark. He explained Schmidt's findings by the intricate structure of the enzyme, in which apparently the bond between protein and the prosthetic group had been destroyed by grinding, thus blocking its activity.

Lyubimenko's hypothesis on the existence of a definite redox potential in the chlorophyll-producing cells was recently confirmed by cytological investigations. V. A. Yakovleva[151] investigated the physicochemical properties of coniferous embryo cells and found that the spruce-seed oxidation rate in endosperm is higher than in pine and larch endosperm.

Goodwin and Owens (1947), Röbeln (?) (1956), Godnev et al. (1959), and Lebedev (1961)[152] observed that in some angiosperms minute amounts of chlorophyll are synthesized in complete darkness. This occurrence is explained by S. I. Lebedev[153] as resulting from a certain degree of heterotrophic nutrition in these plants in addition to their autotrophic nutrition. On the other hand, he suggests that in some plants chlorophyll synthesis in the dark is apparently induced by the presence of a special catalyst. The enzymatic system of chlorophyll synthesis in flowering plants is activated by light. This again confirms Lyubimenko's hypothesis on the enzymatic nature of chlorophyll synthesis and the direct participation of light in this process.

Effect of temperature on chlorophyll synthesis. Most physiologists regarded chlorophyll synthesis as a purely photochemical process, ignoring the temperature factor. Lyubimenko assumed that temperature had a greater significance in chlorophyll synthesis than generally believed. From very early publications it was known that a temperature minimum exists for chlorophyll synthesis within the narrow range of 3 – 11°C,[154] and that the dependence of chlorophyll accumulation on temperature at an equal light intensity can be illustrated by a curve with three principal points: minimum, maximum and optimum.[155] Later, hypotheses advanced by Liro[156] and other scientists on the photochemical synthesis of chlorophyll directly from a hypothetical leucophyll, overshadowed the significance attributed to temperature in the process.

Lyubimenko assumed that temperature — like light — is also a limiting factor. He therefore proceeded to establish with the aid of spectrocolorimetry, the relationship between temperature variations and corresponding changes in the rate of chlorophyll accumulation. He thereby expected to reveal the character of the chlorophyll-accumulation process. The temperature coefficient of the chlorophyll-accumulation rate might show whether photochemical reactions — the rate of which does not change upon a temperature increase of 10° — or catalytic reactions — the rate of which may change considerably with temperature changes — play the decisive role in this process. Investigations in this direction were first undertaken by E. R. Gyubbenet;[157] Lyubimenko's co-worker, and later continued in collaboration with Lyubimenko,[158] The two scientists wished to establish the temperature coefficient of chlorophyll synthesis, the principal points of the reaction and the effect of time. Their observations were carried out on etiolated wheat seedlings.

It was shown that chlorophyll synthesis in etiolated wheat seedlings proceeds within certain temperature limits: synthesis of the pigment started at 2—4°C, reached its maximum at 26—30°C and ceased at about 48°C. The values of the maximum, minimum and optimum temperatures proved to be independent of the length of exposure to light. Any change of temperature greatly affected the course of this physiological process, especially changes from the minimum and maximum temperatues toward optimum. Chlorophyll production was always found to be highest at optimal temperatures, the increment per time unit increasing from 2° to 30°C, and subsequently diminishing with prolonged exposure at a constant temperature. This pattern characterizes the temperature coefficient (Q_{10}) of chlorophyll synthesis (Table 4).

TABLE 4

Temperature (°C)	Temperature coefficient of chlorophyll synthesis in seedlings following exposure to light for different periods of time (in hours)					
	2	8	16	24	48	72
4–14	8.8	15.4	–	7.0	5.4	4.7
10–20	4.7	6.5	8.0	5.0	1.6	–
16–26	4.1	2.9	1.9	1.5	1.4	1.3
20–30	2.6	1.8	1.1	1.1	0.9	–

The temperature coefficient dropped at prolonged exposure to constant temperatures. This was clearly seen between 16—26° to 20—30°. At lower temperatures (4—14° to 10—20°), after an initial increase the coefficient dropped following an 8-hour exposure.

The rate of chlorophyll synthesis changes at constant temperatures. Chlorophyll increment is not infinite even under most favorable conditions. It has been assumed that the principal, and perhaps the only, cause of blockage of the process is the accumulation of chlorophyll in the plant up to a certain optimum above which the rate of the process decreases in time irrespective of temperature. Diminished chlorophyll production per time unit sets in when the pigment concentration reaches about 40% of the maximum.

Up to 30°C the production of chlorophyll is increased together with the increase of temperatures, beginning at 10°C after 48 hours of exposure, at 16 – 20° after 24 hours, and at 26—30°C after 16 hours. Prolonged exposure to light also enhances inhibition of the process. Although, on verifying these findings, E. R. Gyubbenet[159] obtained more accurate results than those obtained in collaboration with Lyubimenko, the general pattern established in the 1920's remained unchanged.

Lyubimenko's theoretical interpretation of these experimental findings was based on his assumption that chlorophyll synthesis takes place according to the following stages: a) leucophyll synthesis; b) transformation of leucophyll into chlorophyllogen; c) transformation of chlorophyllogen into chlorophyll. Since the first two reactions take place in dark and the third in light (being purely photochemical) Lyubimenko assumed that chlorophyll synthesis is influenced by both temperature and light which affect the dark reaction of leucophyll synthesis and its transformation into chlorophyllogen. By designating them dark reactions he did not imply that they took place only in the dark, but that they did not require light. These reactions, being endothermic, can set in only at a certain temperature, the threshold temperature. At temperatures below this threshold the amount of energy absorbed by the seedlings upon illumination is not sufficient to incite these reactions. At temperatures above the threshold, absorption of light energy accelerates the reaction.

Lyubimenko assumed that chlorophyll synthesis is accompanied by other reactions braking the synthesis of the pigment, their rate, however, being negligible under optimal conditions.

On the basis of experimental data Lyubimenko established the **physiological optimum.** In his opinion, the optimum physiological temperature is that at which the physiological process is most rapid and the substances characteristic of the process accumulate in maximum concentration in the shortest time.

Lyubimenko suggested that the decrease of chlorophyll production at temperatures exceeding 30°C may be due to the following: a) stimulation of reactions leading to the formation of a colorless substance from leucophyll other than chlorophyllogen; b) disintegration of the synthesized chlorophyll. The reactions inhibiting the production of chlorophyll begin at a temperature close to optimal. With an increase in temperature the rate of the inhibiting reactions increases, and at maximum temperature it becomes equal to the rate of chlorophyll synthesis; as a result, the production of this pigment gradually drops to zero. However, this objective scientist felt compelled to admit that his experimental data were still inadequate for a full comprehension of the influence of temperature: "A thorough investigation of the influence of temperature on chlorophyll synthesis will be possible only when we are in possession of sufficient data relevant to the rate of these concomittant reactions."[160]

The influence of oxygen on chlorophyll synthesis. Most physiologists were tacitly agreed that chlorophyll synthesis takes place in the presence of oxygen. This conviction was experimentally confirmed by M. D. Dement'ev.[161] Timiryazev[162] and Monteverde[163] also agreed that oxygen is required for chlorophyll synthesis. They showed that the precursors of chlorophyll (protophyllin according to Timiryazev, or protochlorophyll according to Monteverde) are converted by light into chlorophyll as a result of oxidation with atmospheric oxygen. Palladin[164] maintained that chlorophyll synthesis was possible only in an excess of oxygen.

Scharfnagel's[165] investigations, however, introduced certain doubts as to the role of oxygen. To prove that oxygen was required for chlorophyll synthesis he placed etiolated corn seedlings in a nitrogen atmosphere. Oxygen was carefully eliminated from the experimental containers. Nevertheless, chlorophyll still formed in the oxygen-free atmosphere, but protochlorophyll was not quantitatively converted into chlorophyll. The author interpreted the findings as a result of incomplete elimination of the oxygen from the containers or from the vegetal cells.

Norris[166] tested chlorophyll synthesis in etiolated corn seedlings at various oxygen concentrations. He found that the optimal concentration of oxygen lies close to its natural concentration in the atmosphere, i.e., 21%. Chlorophyll synthesis is markedly inhibited only when oxygen is reduced to one-fifth of its normal concentration in the atmosphere.

Although oxygen requirement for chlorophyll synthesis was generally accepted, V.N. Lyubimenko and N.N. Gortikova[167] made a series of experiments to eliminate any doubts possibly arising from Scharfnagel's experiments, and also to find the stage in chlorophyll synthesis at which oxygen is indispensable.

The experiments were carried out with etiolated wheat seedlings. One group of eight-day-old seedlings was placed in an hydrogen atmosphere. The rest were left in the open air at 23°C. After exposure to light for one hour the chlorophyll content of the seedlings kept in the hydrogen atmosphere was somewhat lower than of those kept in the air. The transformation of chlorophyllogen into chlorophyll being very rapid, Lyubimenko assumed that some of the chlorophyll formed by the plants in air had been destroyed before termination of the first hour of illumination. Since this process is continuous, only traces of the pigment could be found after a 24-hour exposure in seedlings kept in a hydrogen atmosphere, whereas a steady increment of chlorophyll was recorded in seedlings kept in air. An hourly increment of chlorophyll was observed by the authors at constant temperature and illumination. Such an induction caused by accumulation of chlorophyll in the plastids was observed earlier by Lyubimenko and Gyubbenet.[168]

The initial synthesis of chlorophyll in the hydrogen atmosphere was possible, according to the authors, through conversion of the chlorophyllogen stored in the seedlings during the dark reaction. After its exhaustion chlorophyll synthesis ceases, apparently because chlorophyllogen synthesis does not take place in an oxygen-free atmosphere.

In another series of experiments after exposure of etiolated wheat seedlings to light for five minutes, thus inducing conversion of chlorophyllogen into chlorophyll, the seedlings were placed in the dark, one group in a hydrogen atmosphere and the other in air. Analysis of the alcoholic extracts prepared at regular intervals revealed that chlorophyllogen is not synthesized in the hydrogen atmosphere, whereas in plants grown in air traces of the substance appear only after 8 hours. Chlorophyllogen accumulated after prolonged exposure. Similar experiments with etiolated corn leaves yielded identical results.

From the data obtained the authors concluded that in chlorophyll synthesis the oxygen is required for the synthesis of chlorophyllogen, and not for the conversion of chlorophyllogen into chlorophyll. Chlorophyllogen is normally converted into chlorophyll in an oxygen-free atmosphere.

This hypothesis enabled Lyubimenko to explain some phenomena described by Liro and Isachenko. They observed that in light, and with complete absence of oxygen, a negligible amount of chlorophyll is formed in dried etiolated leaves. According to Lyubimenko, this observation confirms that the photochemical reaction of chlorophyll synthesis requires no oxygen, whereas the dark reaction of this process takes place only in the presence of oxygen. In his opinion Scharfnagel's observations only confirmed that chlorophyllogen is converted into chlorophyll in the absence of oxygen. Obviously, the leaf tissues were adversely affected by the prolonged growth of the seedlings in the oxygen-free atmosphere, and, therefore, incomplete conversion of chlorophyllogen into chlorophyll was observed by Scharfnagel. Lyubimenko kept etiolated corn seedlings 24 hours in a hydrogen atmosphere. The amount of protochlorophyll subsequently synthesized by these seedlings in air was smaller than that formed by leaves which had been kept in air for the same period (24 hours). It was concluded that leaves do not lose their ability to form chlorophyllogen in the presence of oxygen after being kept in a hydrogen atmosphere.

Guided by these findings, Lyubimenko and Gortikova suggested the following stages in the synthesis of chlorophyll:

Protein A + chromogen = leucophyll
Leucophyll + O_2 = chlorophyllogen
Chlorophyllogen + light (or enzyme) = chlorophyll

The authors, however, were aware that: "This scheme is undoubtedly mainly hypothetical, offering at best a working hypothesis for further biochemical and physiological investigations". [169]

Oxidation-reduction regime of the cell and transformations of chlorophyll. Since the conversion of leucophyll into chlorophyll represents per se an oxidation reaction, Lyubimenko[170] assumed that chlorophyll synthesis depends to a considerable extent on the intensity of the oxidation-reduction reactions in the cellular protoplasm. Chlorophyll synthesis stops at low oxidation intensities, and excessively energetic oxidations are also unfavorable for its synthesis. If the latter occurs, chlorophyll accumulation is hindered by the rapid destruction of the pigment induced by the activity of oxidation enzymes. Furthermore, Gortikova[171] assumed that the redox potential contributes to the conversion of chlorophyllogen into chlorophyll: in darkness this potential is lowered and conversion of chlorophyllogen into chlorophyll is arrested.

Later, N.N. Gortikova and D.I. Sapozhnikov[172] attempted the quantitative estimation of the oxidation intensity governing chlorophyll synthesis. A series of experiments was carried out for determination of the optimal redox potential required for chlorophyll synthesis by etiolated wheat seedlings in aerobic and anaerobic conditions, in various oxidation-reduction systems and at various pH values of the medium. In order that the chlorophyllogen might be completely eliminated the seedlings were preliminarily exposed to light. The seedlings were then placed in a buffer solution (pH 6.98) supplemented with ascorbic acid. It was observed that in solutions containing the acid in small or moderate concentrations chlorophyll formed in greater quantities than in the control group (kept in the buffer solution alone); very little chlorophyll was formed in solutions containing the acid in high concentrations. The authors observed that chlorophyll synthesis depends on molecular oxygen which, in artificial conditions, can be replaced by another oxidation-reduction system (rH) not lower than 18—20). These observations as well as those of Yablokova[173] strengthen Lyubimenko's hypothesis on the negative effect of overoxidation.

From the experimental findings Lyubimenko concluded that both synthesis and disintegration of chlorophyll and of other leaf pigments are linked to changes of the redox potential in the vegetal cells.[174] When noting the steadily increasing activity of the peroxidase enzyme concomitant with the aging of the leaves, Lyubimenko ascribed this increase to a well-defined characteristic of the cellular oxidation activity. This activity is rather limited in young leaves since their pigment system is almost colorless, i.e., is in a reduced state. In time the enzymatic activity increases and the gradually oxidized pigments acquire their color. In fall the pigments again become colorless substances as a result of oxidation. The stable concentration of pigment in summer leaves is explained by a certain optimal magnitude of the oxidation potential leading to a state of equilibrium between the rate of oxidation of protochlorophyll into chlorophyll and of chlorophyll into a colorless product of oxidation. In Lyubimenko's opinion this equilibrium is regulated by a protective, reducing substance, which he designated antioxidase and which presumably appears in the chloroplasts.

Lyubimenko[175] pointed out that what happened to chlorophyll after synthesis was still unknown. However, recent investigations established that he was wrong to discard the hypothesis, advanced by Sachs and Wiesner in the 19th century, that chlorophyll is constantly synthesized and disintegrated. Lyubimenko shared Willstätter's opinion that chlorophyll, once formed in the chloroplasts, is considerably stable, and that its content changes or decreases only under exceptionally adverse conditions, in aging leaves. All these speculations lasted until paper-chromatography and labeled-atoms techniques were introduced. These new methods made it possible to differentiate, in living plants, between recently formed chlorophyll molecules and those synthesized earlier, and enabled the detection of synthesis and disintegration patterns of pigment molecules.

The first to observe chlorophyll renewal in green plants were Roux and Husson.[176] They were followed by a series of communications on the rate of this process (Turchin et al.; Becker and Sheline; Shlyk[177]). The results obtained by these scientists decisively established that intensive renewal of chlorophyll molecules takes place in the leaves, and that the process is very sensitive to changes in conditions of both the plant and the environment.

6. QUANTITATIVE VARIABILITY OF CHLOROPHYLL

Lyubimenko always maintained that an accurate estimate of the chlorophyll concentrate can be possible only after determinations are made of the chlorophyll content in the chloroplasts. The technical facilities required for this purpose, however, had not yet been devised. Haberlandt[178] and Griffon[179] suggested plastid counts of the leaf parenchyma and visual estimation of their coloration intensity. This method gave only an approximate estimate of the pigment concentration in the plastids and its content in the leaves.

As knowledge of chlorophyll became more extensive and the methods of its extraction improved at the beginning of the 20th century, a fairly accurate determination of its content in the plant became possible. Lyubimenko's first investigations in this direction were conducted in 1908—1910.[180] After extracting all the chlorophyll from a plant he accurately determined its quantity by spectrocolorimetry. This method takes advantage of certain optimal properties of these pigments, such as the intensity of the first absorption band of chlorophyll; the experimental solution is compared with that of a previously prepared standard solution of known concentration.

Although spectrocolorimetry was a well-known tool in science, botanists made almost no use of it, possibly because there was no adequate apparatus for their purposes. In 1907 Lyubimenko devised a special apparatus for quantitative analysis of chlorophyll. The disadvantage of this instrument was that it required an ample supply of known, standard solution. In 1913 Monteverde and Lyubimenko[181] built a new apparatus (Figure 4) which, after some improvements,[182] was readily adopted by researchers. Spectrocolorimetry was, in Lyubimenko's opinion, a most convenient method for mass investigation and he used it successfully in many of his quantitative analyses of chlorophyll and other plant pigments. In Java he thus managed to determine the chlorophyll content of 200 tropical species in less than 2 months. One of the advantages offered by spectrocolorimetry is that it enables the direct determination of chlorophyll traces in alcoholic extracts, without preliminary isolation of the yellow pigments.

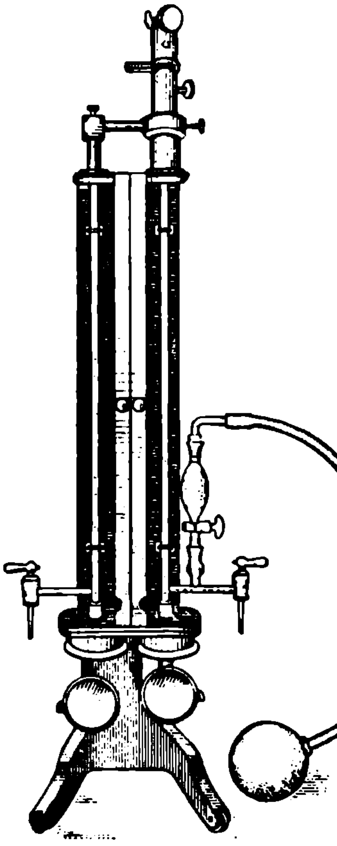


FIGURE 4. Spectrocolorimeter built by Lyubimenko and Monteverde for quantitative analyses of plant pigments

Lyubimenko was the first to determine the chlorophyll content of several hundred species. A summary of this work and certain conclusions were published in his article: "The Chlorophyll Content of Plants from Various Geographical Latitudes" (O kolichestve khlorofilla u rastenii razlichnykh geograficheskikh shirot).[183] More detailed data on the variability of chlorophyll content are related in his doctoral thesis: "Conversion of Pigments in Plastids of Living Plant Tissues" (O prevrashchenii pigmentov plastid v zhivoi tkani rasteniya).

More than 650 species from various geographical latitudes and habitats were analyzed. Observations in the botanical gardens of Leningrad, Nikitsk and Buitenzorg disclosed extreme variations of the chlorophyll content in different representatives of the plant kingdom, depending on the latitude of their habitat (Table 5).

It was observed that at the Leningrad latitude (60°N) the average chlorophyll content of most plants fluctuates between 2.1 and 3 g/kg. Certain deviations from this average are manifested in photophilous species with a lower chlorophyll content on the one hand and in sciophytes with a somewhat higher content on the other. At the Crimean latitude (45°N) an average chlorophyll content could be established in only half the number of plants, since there are more photophyte and sciophyte plants than at the

Leningrad latitude. The chlorophyll content of the sciophytes reaches 5 g/kg. The tropical vegetation of Java (6°S) consists mainly of photophytes and sciophytes. Only one-quarter of the species has an average chlorophyll content. Advancing southwards the plants with an average chlorophyll content become less abundant and the tendency toward differentiation of the vegetation into photophytes and sciophytes becomes more marked. The chlorophyll content is diminished in the first group, occupying the upper story, and increases in the second group, located in the lower story.

TABLE 5

Chlorophyll content, in g/kg of fresh leaves	Number of plant species, %		
	60° N lat.	45° N lat.	6° S lat.
From 0.8 to 1.0	1.3	0.7	2.5
From 1.1 to 2.0	15.5	26.5	36.0
From 2.1 to 3.0	77.5	50.5	27.5
From 3.1 to 4.0	5.7	16.5	16.0
From 4.1 to 5.0	0	5.8	13.0
From 5.1 to 6.0	0	0	4.0
From 6.1 to 7.0	0	0	1.0

Lyubimenko's approach to the interpretation of his data was that of a botanist and Darwinist. The statistical data only provided the tools by means of which the biological meaning of the phenomena could be disclosed, namely, the adaptation of plants and their physiological functions in relation to environmental factors, especially light and temperature.

"In the north", wrote Lyubimenko, "where the climate is rather cold, the plants need plenty of light and a strong system for the accumulation of energy; therefore sciophytes are rather scarce, being unable to take advantage of the deeply shaded sites. The maximal chlorophyll content of sciophytes is thus relatively small. That is why competition for light is very intense in the northern latitudes. The plant not only needs space but also good illumination.

"In temperate latitudes, where light deficiency can be partly compensated by heat, light is less important. Therefore, plants can thrive in shaded places by increasing their light-collecting intensity of the assimilation apparatus and competition for space is more important.

"Finally, in the Tropics competition for space comes first; that for light being of secondary importance. Since light deficiency is amply compensated by heat, plants can thrive in most of the shady places. That is why most representatives of the various categories of sciophytes are encountered in the Tropics, their absolute chlorophyll content reaching unusually high values.

"... It follows that the intensity of light absorption by the assimilation apparatus of green plants in various geographical latitudes is so coordinated as to compensate heat deficiency by light in the North, and light deficiency by heat in the Tropics."

Lyubimenko opposed the widely adopted theory that the energy of photosynthesis depends on the light absorption intensity of the chlorophyllous apparatus. In his opinion: "the chlorophyll content of the leaf mainly indicates a specific adaptation to a certain light intensity. The energy of photosynthesis, as expressed in terms of the quantity of dry matter accumulated, is apparently determined by the rate of elaboration of the primary assimilates, which in turn depends on the properties peculiar to the plant species."[185]

The difficulties encountered when undertaking accurate determinations of the chlorophyll content in plants were repeatedly mentioned by Lyubimenko. He emphasized that various factors of nutrition, illumination, water supply, age of individual representatives of the same species, and different contents and proportions of chlorophyll *a* and *b* should be taken into consideration when attempting such determinations. He himself did his best to reckon with these factors and was very impressed with the investigations of the German chemist R. Willstätter.[186] Willstätter and Stoll established the content of chlorophyll *a* and *b* in many higher plants of different ages and at various light intensities. They found that the chlorophyll *a* content is 2.5—3.5 times as large as that of chlorophyll *b*. The total chlorophyll content of most plants, with the exception of the "yellow" variants, is 0.7—1.3% of the dry mass of the leaves. In Lyubimenko's opinion the average content does not depend on the geographical latitude, although the amplitude of fluctuations is much wider in the Tropics than in the temperate zone.

Chlorophyll content was also investigated by Garder, Seybold and Egle, Emerson, etc.[187] They established that leaves of sciophytes contain both more chlorophyll in general and more chlorophyll *b*. This led Egle[188] to designate chlorophyll *b* "the typical shadow pigment".

Using Lyubimenko's rich experimental data on the quantitative variability of chlorophyll, the Soviet biochemist A. V. Blagoveshchenskii calculated the average chlorophyll content of 360 species of higher plants, belonging to 20 families. He tabulated the plants in order of increasing chlorophyll content, thus revealing an interesting phenomenon in the evolution of the plant kingdom. The lowest content of chlorophyll is manifested in leaves of gymnosperms of the family Cycadea, formerly an enormous group widely distributed in the Carboniferous period. Little chlorophyll was also found in leaves of Coniferae, also a very ancient group of gymnosperms with archaic structural features.

On the other hand, Gramineae, Leguminosae and other contemporary, widely distributed species are considerably richer in chlorophyll. Gramineae comprises over 4500 species distributed in all latitudes, especially in the Tropics. It is represented exclusively by grasses which may reach a height of 20m and a diameter of 30cm. The contemporary cereals possess the following properties: unusual ability for vegetative reproduction; high resistance to drought and cold; resistance to soil salinity and acidity; ability of the root system to survive temporary anaerobic conditions, such as caused by flooding. All these properties encouraged the wide distribution of cereals over vast territories, including areas unsuitable for many other plants. Although their structure is simplified, the plants have grown more viable. Blagoveshchenskii writes: "There is no doubt that this viability is very closely related to the high chlorophyll content recorded by Lyubimenko to be in cereals".[189] The same can be said of Leguminosae, the chlorophyll content of which approximates that of the cereals. Leguminosae comprise almost 15,000 species distributed throughout the world and widely represented by trees, shrubs, creepers and grasses. With regard to Liliaceae (the ancestors of cereals) and Rosaceae (the ancestors of Leguminosae) occupy an intermediate position with regard to their chlorophyll content, corresponding to their taxonomic position among angiosperms.

It follows that the chlorophyll content of progressively evolving families is higher than that of ancient, partly extinct families.

Lyubimenko was the first to determine the chlorophyll content of aquatic plants, including marine algae.[190] For these investigations he selected the 30 largest representative species encountered in the Black Sea algal flora (collected in the vicinity of Yalta and Sevastopol) as well as Mediterranean algae from the southern coast of France (the Bagnoles Biological Station).

Spectrocolorimetric determinations revealed that the chlorophyll content of algae, of all colors, is much lower than that of terrestrial and submarine higher plants, and that the deeper the habitat, the lower its chlorophyll content. Considering the chlorophyll content of the terrestrial plant Ailanthus glandulosa as 100%, Lyubimenko found that the chlorophyll content of green algae equals 24 and 16%; of brown algae, growing at a greater depth — 20, 18, and 10%; and of red algae living at an even greater depth — 11, 10, 7 and even 2%. The chlorophyll content of leaves of the marine grass Zostera marina, a higher plant growing at the same depths as the brown and red algae approximates that of terrestrial sciophytes, i.e., it is considerably higher than that of algae.

Willstätter[191] was also of the opinion that algae contain less chlorophyll than terrestrial plants. Seybold and Egle [192] obtained different results. They found that the chlorophyll content of most algae approximates that of higher plants. The reason for such a discrepancy is still unknown.

Quantitative determinations by spectrocolorimetry of the red pigments (phycoerythrin) were conducted on a series of red algae taken from various depths. These revealed that the deeper a given algal species lives, the greater its chlorophyll and phycoerythrin content. Thus, photophilic and sciophilic forms are also found among algae.

Lyubimenko investigated changes induced by age in the pigment content of plastids of terrestrial plants. He found that young leaves contain less chlorophyll than old ones. With age, the chlorophyll content of the leaf again decreases until complete destruction of the pigment is reached. He also observed that the decrease of chlorophyll is slower than that of the yellow pigments. The yellow pigment reaches its maximum in young leaves. The ratio of chlorophyll to yellow pigments, therefore, increases with age.

7. CHROMATIC ADAPTATION

Lyubimenko traced and studied the spectra of green, red and brown marine algae. The patterns that he revealed confirmed the theory of Engelmann and Oltmanns that the zonal distribution of algae depends on the intensity and quality of the light. Green algae prevail in shallow waters, red algae in deep waters, and brown algae at average depths. Engelmann[193] attributed this distribution to an adaptation to the quality of light and to its dominant color. Accordingly the most effective absorption of the light penetrating through the water is achieved when the color of the plant pigment is complementary to that of the environment. He designated this phenomenon "chromatic adaptation". Engelmann explained the variations of plastid pigmentation by the presence of supplementary pigments, in addition to chlorophyll, namely phycocyan and phycoerythrin. Oltmanns[194] maintained that the vertical distribution of algae is not a function of the dominant color of the light but of its intensity. This controversy lasted many years. Harder's suggestion[195] that both the color and the intensity of light are significant in the process of adaptation of algae to various environmental conditions, seems more acceptable since the light at a depth of 20m, for instance, is devoid of both red and violet rays and represents only the 20th part of ordinary light. Lyubimenko[196] was therefore right in considering the deep-sea algae as typical "sciophytes" rich in pigment. In his opinion, the pigment composition of these algae is not only derived from the poor illumination but also from the relatively low intensity of the red and violet rays.

According to Lyubimenko's observations, chromatic adaptation consists in the increased pigment concentrated in the plastids concomitant with the depth of the habitat, and this occurs to the same extent in all algae, irrespective of color. It was established that the scanty amount of chlorophyll in red and brown algae, compared with that in green algae and higher aquatic plants, is compensated by the accumulation of phycoerythrin and phycoxanthin. The presence of these auxiliary pigments, enhancing the absorption of green rays by plastids, was considered by Lyubimenko to be the final chromatic adaptation of the algae to the poor light provided by the terminal red rays. In his opinion, chromatic adaptation was achieved by the red algae in two ways: on the one hand by a quantitative change of the pigment in the plastid stroma and on the other, by a regular change in the proportions of the individual pigments. The dependence of pigment concentration on the depth of habitat — in the same species — is considered by Rabinowitch[197] as a typical ontogenetic adaptation to light intensity, whereas the increased ratio of phycoerythrin to chlorophyll with the concomitant depth of habitat — in various species — is defined by the same author as a typical example of phylogenetic adaptation to light.

Lyubimenko[198] studied the chromatic adaptation of algae in connection with photosynthesis. In 1928 he obtained, in collaboration with Z. P. Tikhovskaya[199] the first definite data on the connection between the change of algal coloration and carbohydrate nutrition of algae. Lyubimenko and Tikhovskaya found that the plastid pigmentation has no effect on photosynthesis up to a depth of 50m, and that an increased pigmentary concentration can secure sufficient energy for photosynthesis at the same variations in the spectral composition of light as those usually occurring at great depths. Irrespective of the plastid pigmentation two main biological groups are distinguished according to the intensity of photosynthesis: shallow-water and deep-water algae. There are also some intermediate transitional forms.

8. FOSSIL CHLOROPHYLL

Lyubimenko also published works on fossil chlorophyll. He investigated sapropels, i.e., organic or lacustrine deposits, as well as marine silts mainly consisting of organic debris of aquatic organisms (including algae).[200]

The presence of a green substance in slimy deposits was first mentioned by Ehrenberg (1841–1842). The optical properties of alcoholic extracts of this pigment were first mentioned by Post (1861–1862), Gignier (?) (1880), Hoppe-Seyler (1886–1889) and Frew (1904).[201] Lyubimenko noticed that in dying terrestrial plants chlorophyll is readily and rapidly oxidized by atmospheric oxygen and therefore cannot participate in the formation of sapropels. Only algal chlorophyll can survive in such sediments since oxidation processes in algae are considerably weaker than in terrestrial plants. Lyubimenko wrote: "It is most probable that the fossil chlorophyll present in sapropel is solely derived from algae, their oxidation apparatus being less intensive than that of mosses and higher plants."[202] Light and oxygen have limited access to the bottom of water bodies, the plastid pigments are protected by the algal enzymes from disintegration through oxidation, thus retaining their chemical integrity for a long time.

Lyubimenko found that the spectrum of alcoholic chlorophyll extracts from marine sediments or lacustrine sapropel from the Tertiary, Glacial and Recent periods closely approximates the spectrum of diatoms, of which these deposits consist. Alcoholic chlorophyll extracts of marine deposits treated with barium hydroxide revealed that the main properties of this green pigment are preserved in fossil chlorophyll. Carotenoids were found in benzene extracts. Lyubimenko subsequently isolated crystalline and amorphous carotenoids. He maintained that the good preservation of organic compounds in sapropels for hundreds, thousands and even millions of years can be explained by the favorable conditions (darkness, absence of oxygen, low temperature (about 0°C), etc.) prevailing at great depths.

9. CHLOROPHYLL AND HEREDITY OF PLANTS

Lyubimenko was also interested in the inheritance of certain properties of plant pigments. At the experimental tobacco plantation of the Nikitskii Botanical Garden in 1916, in collaboration with the manager of the plantation, he carried out a series of experiments which proved to be of both theoretical and practical significance.[203] The inheritance from generation to generation of a constant chlorophyll content in diverse varieties and races of tobacco (Nicotiana tabacum) was investigated.

The subject interested him because the "ripening" of tobacco leaves implies disintegration of the chlorophyll and its replacement by yellow pigments. Tobacco varieties poor in chlorophyll are preferable to those with a rich chlorophyll reserve because the former ripen rapidly and uniformly, whereas the latter ripen slowly and late. One of the aims of tobacco selection, therefore, is to breed lines with rapidly ripening leaves in order to simplify and accelerate drying.

First, Lyubimenko tried to find whether a constant chlorophyll content is inherited in normal green plants. There were no data on this subject in the literature, the only observations published being those of Correns[204] and Baur[205] who investigated the inheritance of pigments (chlorophyll content) in hybrids obtained from crosses with albinos, plants with variegated leaves and normal green plants. These experiments showed that the following three alternatives of color inheritance are possible: 1) color transmission according to Mendel's law; 2) inheritance of the mother's color only; 3) inheritance of both paternal and maternal color, but not according to Mendel's law.

In Lyubimenko's opinion, these results disproved the universality of Mendel's law on color heredity and proved that in the plant cell "along with the ordinary transmission of genes through the nucleus.... there is also a transmission of some elements through the protoplasm."[206] In other words, heredity is carried by both genes and other parts of the cell.

Color intensity of hybrids and their progeny were earlier estimated by sight, whereas Lyubimenko conducted accurate determinations by spectrocolorimetry. The experiments were carried out on normal green tobacco plants. Hybrids were analyzed up to the third generation, or even to the fifth. It was found that in crosses between races with various amounts of chlorophyll the forms poor in chlorophyll were not always dominant, since they were found only in 4 out of 21 combinations. In 13 hybrids the chlorophyll content was the arithmetic mean of the parental contents. In three crosses the chlorophyll content was higher than in both parents. In one instance the chlorophyll content was equal to that of the parent with the lower chlorophyll content.

It was observed that the chlorophyll content index of the second and third hybrid generations actually follows Mendel's law, but its recurrence being only occasional, it should not be considered a regular pattern. The same was observed in the experiments with plants with variegated leaves; most hybrids already inherited the maternal or paternal chlorophyll content in the first generation, or exhibited a generally greater chlorophyll content in comparison with that of the parental forms. Increased chlorophyll content in hybrids was also observed by Schull[207] who crossed pale-gray races of Melandrium. Lyubimenko ascribed great theoretical significance to this phenomenon, linking it to the inner factors of chlorophyll synthesis. In his opinion, the chlorophyll content of plastids depends on the properties of the plastids themselves and on inner physiological properties of the cells and tissues, especially on their oxidation regime. Crossing a race with a high oxidation regime with one possessing a low oxidation regime may result in a combination favorable for chlorophyll synthesis. The hybrids of such a cross may therefore seem to be richer in chlorophyll than their parents.

Lyubimenko came to the conclusion that the amount of chlorophyll, or better its concentration in the plastids, is a constant hereditary feature. This theory was substantiated by L. P. Breslavets[208] who investigated the hereditary properties of yellow pigments in the chromoplasts of the corolla of Tropaeolum majus.

In one of his last publications, in collaboration with O. A. Shcheglova and N. N. Gortikova,[209] Lyubimenko outlines a project to investigate the hereditary transmission of chlorophyll content and, in addition, to ascertain the genesis of this pigment and its quantitative ratio to carotenoid pigments.

In 1930 it was discovered that the chlorophyll constituents, carotene and phytol, are chemically closely related. Lyubimenko assumed that the processes of their synthesis have much in common. Quantitative studies of the ratios of yellow to green pigments in various specimens of one species (i. e., within the limits of one genotype), established that the amount as well as the molecular relationships are not stable; in fact, their variability revealed quantitative fluctuations of each pigment as if independent of the other pigments. In hybrids Lyubimenko obtained similar results although there were some cases in which — compared with the parents — a definite increase or decrease of the pigment concentration was found. They may be explained by great changes in the genotype of the hybrid. Interspecific crosses also led to independent changes (though only within the limits of the pigment ratios) such as phenotypic variations within one species. However, the prevalence of chlorophyll over xanthophyll and of the latter over carotene remained unchanged.

These observations led the authors to the conclusion that the pigment components of natural chlorophyll are not linked in proportions that would correspond to a definite molecule of a single complex compound. At the same time, these proportions are not arbitrary. The amount of carotene is always the smallest and that of xanthophyll average, whereas the chlorophyll forms the bulk. This pattern of pigment distribution is ascribed by some scientists to the probable formation of all chromophore groups from the same initial, colorless compound (leucophyll) through rearrangement of its molecule.

Lyubimenko's hypotheses on the mechanism of hereditary transmission of coloration in plants are as follows. The amount of green and yellow pigments of the plastids is determined by the amount of the protein-chromogen complex (leucophyll) accumulated in the cell. If the plastid is inherited, its protein, designated factor Q, is also inherited. In the absence of this factor no pigment can arise and complete albinism will ensue. Another hereditary factor of this complex is chromogen, known as factor C. Its content in the plant cell is determined by the intake of nutritional substances (carbohydrates, Mg, iron, etc.) and by a specific enzyme inducing chromogen synthesis in the dark, which is designated by the letter C. According to Lyubimenko, transformation of chromogen into colored prosthetic groups of the pigment takes place through oxidation with the help of oxidative enzymes of the protoplasm. This factor is designated by the letter O. Unlike factor C, factor O is not specific to chlorophyll synthesis.

The quantitative variations of factors C and O determine the depth of tone of the color, but do not lead to considerable changes in the ratio of the green to yellow pigments. A decrease in the intensity of normal green pigment can lead to albinism. Three factors are responsible for albinism: 1) absence of factor C; 2) deficiency of factor O; 3) surplus of factor O. As far as is known, not only the intensity of the color but also the plastid color itself changes. Lyubimenko suspected the existence of yet another specific factor — factor X — of the chlorophyllase type which, during chlorophyll synthesis, is responsible for the esterification of the acid groups of chromogen with methanol and phytol at the expense of the carotenoids. In the absence of chlorophyllase, transformation of leucophyll may cease at the stage of carotenoid formation, the plastids then acquiring a yellow tint. The same coloration appears if there is very little chlorophyllase, when carotenoids are more abundant than chlorophyll.

It follows that the intensity of plant coloration and the color itself are determined by four hereditary factors, Q, C, O, X, of which Q, C, X are required for chlorophyll synthesis. Factor O is of a general character, determining the course of the oxidation reaction in general and creating the internal conditions for chlorophyll synthesis. Hence, plant coloration mainly depends on the enzymatic make up. When the parental forms have an inadequate oxidation regime, for instance one parent bearing a high oxidation potential and the other a very low one, the resulting hybrids may be endowed with a favorable average potential promoting a greater pigment accumulation than in the parent plants.

The influence of environmental conditions such as light, temperature, nutritional substances, etc. on plastid coloration have already been discussed in detail. In his above-mentioned work, Lyubimenko stressed the dual role of light, as a photo-oxidant of chlorophyllogen and as a determinant factor in the carbohydrate metabolism of plants (carbohydrate synthesis) and the course of oxidation processes. Temperature directly affects the rate of enzymatic activity in chlorophyll synthesis as well as enhances or suppresses the intensity of oxidation processes in the protoplasm.

Thus, plastid coloration depends on a single complex of green and yellow pigments linked to each other and to the protein of the stroma in certain molecular proportions. Under normal internal and external conditions, this complex is inherited by the plants as an integral unit, i. e., in the form of one hereditary property. "It goes without saying," writes Lyubimenko, "that the hereditary factors (Q, C, O and X - E. S.) cannot be identified with the true genes found in the nuclear chromosomes of the cell. Ours are physiological factors, directly participating in the synthesis of pigments... If we proceed from the concept that the gene is a particle completely independent of living matter endowed with a definite physiological activity, then this activity will be determined not only by the quality of the gene but by the internal conditions under which it will take place... Studies of the heredity of plastid pigmentation are a great advantage since they enable us to follow not only the amount of respective pigments but also the biochemical processes involved in their production, i. e., they enable us to approach the study of the physiological activity of the genes."[210]

This last work on the content of various pigments in interspecific hybrids of Nicotiana, was defined by Lyubimenko as "the first step in the physiological analysis of heredity." And indeed, this was the first attempt to give a general biological analysis of some aspects of the inheritance of certain physiological features. The author paid much attention to the influence of external conditions on the plant organism, its general physiological state and the character of cell metabolism. His premature death interrupted the investigations. It is to be regretted that the study of heredity and variability of physiological features in plants is not being pursued with the enthusiasm of its initiator.

Twenty-five years have elapsed since the untimely death of this energetic, cheerful man, who persevered in the study of the most complicated processes in plant physiology. V. N. Lyubimenko strengthened the most valuable traditions of his predecessors in Soviet science, A. S. Famintsyn, K. A. Timiryazev, M. S. Tsvet, all of whom aimed at elucidating the nature of photosynthesis and the role of chlorophyll in the process.

V. N. Lyubimenko combined technical perfection with cautious experimental investigation and a broad theoretical and practical approach. Continuing the investigations initiated by K. A. Timiryazev on the photosynthetic make-up of plants, Lyubimenko brilliantly solved the problems of the optical and chemical properties of chlorophyll and the production and transformation of this pigment in the plant, in addition to investigating it from the aspect of evolutional biology. His well-founded hypotheses on the structure and synthesis of chlorophyll offered a new means of approach to the investigation of the origin and evolution of photosynthesis and the inherent production of organic substances resulting from plant metabolism.

The investigation of chlorophyll and its role in photosynthesis has advanced considerably during the last decades. Not all concepts and hypotheses of the past have been substantiated, but there is no doubt that V. N. Lyubimenko, the outstanding Soviet plant physiologist, made no small contribution to the progress of modern science.

Notes

[1] Gyubbenet, E.R Obzor rabot V. N. Lyubimenko po pigmentam plastid (A Review of V. N. Lyubimenko's Works on Plastid Pigments). - Trudy Botanicheskogo Instituta AN SSSR, Seriya 4(4):24. 1940.

[2] Apud: Lyubimenko, V. N. and V. A. Brilliant. Okraska rastenii (Coloration of Plants). – Leningrad, Gosizdat. 1924.

[3] Pelletier J. et J. Caventou. Sur la matière verte des feuilles. – Ann. Chem. et phys., 1818.

[4] Kayser, H. G. J. Handbuch der Spectroscopie, Bd 1-6. Leipzig, 1900-1912.

[5] Timiryazev, K. A. Otchet po zagranichnoi komandirovke iz Parizha ot 10 fevralya 1870 (Account of a Mission to Paris from 10 February 1870). Collected Works, Vol. 2:337-358. –Moskva, Sel'skhozgiz. 1937.

[6] Reinke, J. Photometrische Untersuchungen über die Absorption des Lichtes in den Assimilationsorganen. – Bot. Ztg, Jg. 44, 1886, S. 101, 177, 193, 209, 225, 241.

[7] Pringsheim, N. Über das Hypochlorin und die Bedingungen seiner Entstehung in der Pflanze. –Monatsber. Berliner Acad. Wiss., November 1879.

[8] Nentskii, M. V. O biologicheskikh sootnosheniyakh mezhdu krasyashchim veshchestvom list'ev i krovi (Biological Relationship between the Leaf Pigment and Blood). –Arkhiv Biologicheskikh Nauk, Vol. 5:305. 1897.

[9] Tsvet, M. S. Gemoglobin i khlorofill. V kakom napravlenii zhelatel'no izuchenie poslednego tela (Hemoglobin and Chlorophyll. The Correct Study of Chlorophyll). –Izvestiya Sankt Peterburgskoi Biologicheskoi Laboratorii, 2(3):61. 1898.

[10] Ibid., p. 63.

[11] Tsvet, M. S. O khloroglobine (Chloroglobin). —Trudy Sankt Peterburgskogo Obshchestva Estestvoispytatelei, Vol.30, No. 1(8):346. 1899.

[12] Tsvet, M. Sur la constitution de la matière colorante des feuilles. La chloroglobine C.n. acad. sci., Paris, t. 129, 1899, p. 607-610; M. Tsvet. Das Chloroglobin, Bot. Centralblatt, Bd 81, 1900, S. 81.

[13] Monteverde, N. O "khloroglobine" i ego otnoshenii k khlorofillu ("Chloroglobin" and Its Relation to Chlorophyll). – Botanicheskie Zapiski Sankt Peterburgskogo Obshchestva Estestvoispytatelei, No. 15: 293-298. 1900.

[14] Czapek, F. Kohlensäure-Assimilation und Chlorophyll. – Bot. Ztg., Jg. 58, 2 Abt., 1900. S. 69.

[15] Tsvet, M. O prirode khloroglobina (The Nature of Chloroglobin). – Trudy Sankt Peterburgskogo Obshchestva Estestvoispytatelei, Vol. 31, No. 1(4):161. 1900.

[16] Tsvet, M. S. Fiziko-khimicheskoe stroenie khlorofill'nogo zerna (The Physicochemical Structure of Chlorophyll Granules). — Trudy Obshchestva Estestvoispytatelei pri Kazanskom Universitete, 25(3), 1901.

[17] Tsvet, M. S. O novoi kategorii adsorbtsionnykh yavlenii i o primenenii ikh k biokhimicheskomu analizu (A New Category of Adsorption Phenomena and Their Use in Biochemical Analysis). – Trudy Varshavskogo Obshchestva Estestvoispytatelei, Otdel Biologicheskii, Vol. 14:20-39. 1903; by the same author: Khromofilly v rastitel'nom i zhivotnom mire (Chromophylls in the Plant and Animal Kingdom). – Warszawa, 1910.

[18] Willstätter and A. Stoll. Untersuchungen über Chlorophyll. Berlin, 1913.

[19] Lyubimenko, V. N. O svyazi khlorofilla s belkami plastid (The Chlorophyll Bond with the Plastid Proteins). –Dnevnik pervogo Vsesoyuznogo s''ezda russkikh botanikov v Petrograde, pp. 46–47. Peterburg. 1921.

[20] Lyubimenko, V. N. Issledovanie pigmentov plastid, II soobshchenie: svyazi khlorofilla s belkami plastid (Studies of Plastid Pigments.. 2nd Communication: The Chlorophyll Bond with the Plastid Proteins). – Izvestiya AN SSSR, Seriya 6, Vol. 17. 1923.

[21] Ibid., p. 147.

[22] Sapozhnikov, D.I. Fotoreduktsiya azotnokislogo serebra natural'nym khlorofillom (fitokhromoproteid plastidy) (Photoreduction of Silver Nitrate by Natural Chlorophyll (the Phytochrom-Protein of Plastids)). – Doklady AN SSSR, 61(3):561-564. 1948.

[23] Nezgovorov, L. A. Razrushenie khlorofilla plastid i zashchitnaya rol' belkov tsitoplazmy (Disintegration of Plastid Chlorophyll and the Protective Role of Cytoplasmic Proteins). – Doklady AN SSSR, 90(6): 1175-1178. 1953.

[24] Zirkle, C. The Structure of the Chloroplast in Certain Higher Plants. – Amer. J. Bot., Vol. 13, p. 301-341. 1926.

[25] Sapozhnikov, D. I. Razdelenie i kolichestvennoe opredelenie fitokhromov plastidy (Isolation and Quantitative Determination of Plastid Phytochromes). – Trudy Botanicheskogo Instituta AN SSSR, Seriya 4(8): 140-163; 1951.
Sapozhnikov, D.I. Novye nablyudeniya nad khlorofillom (Recent Observations on Chlorophyll). – Ibid., No. 9:123 – 131. 1953.
Sapozhnikov, D.I. and T.G. Maslova. K voprosu o sostoyanii khlorofilla v list'yakh zelenykh rastenii (The State of Chlorophyll in the Leaves of Green Plants). – Ibid., No11:97 – 115. 1956.

[26] Krasnovskii, A.A. and G.P. Brin. O prirode aktiviruyushchego deistviya osnovanii na reaktsiyu obratimogo fotokhimicheskogo vosstanovleniya khlorofilla i feofetina (The Character of the Activating Effect of Bases on the Reversible Photochemical Reduction of Chlorophyll and Pheophytin). – Doklady AN SSSR 89(3):527 – 530. 1953.

[27] Lyubimenko, V. N. and N. N. Gortikova. O roli kisloroda v protsesse zeleneniya (The Role of Oxygen in Chlorophyll Synthesis). – Trudy Botanicheskogo Instituta AN SSSR, Seriya 4(2):113-148. 1926.

[28] Noack, K. Der Zustand des Chlorophylls in der lebenden Pflanze. – Biochem. Zs., Bd 83, S. 135. 1927.

[29] For more details, see: Rabinowitch, E. Fotosintez (Photosynthesis), Vol.I pp.384-397. [Russian translation. 1951.] Gyubbenet, E.R., Rastenie i khlorofill (The Plant and Chlorophyll), pp. 18-53. – Moskva-Leningrad. 1951.

[30] French, C. S. The Chromoproteins of Photosynthetic Purple Bacteria. – Science. Vol. 88, N 2272, p. 60-62. 1938.

[31] Krasnovskii, A.A., K.K. Voinovskaya, and L.M.Kosobutskaya. Priroda estestvennogo sostoyaniya bakteriokhlorofilla v svyazi so spektral'nymi svoistvami ego kolloidnykh rastvorov i tverdykh plenok (The Natural State of Bacteriochlorophyll in Relation to the Spectral Properties of Its Colloidal Solutions and Solid Films). - Doklady AN SSSR, 85(2):389-392. 1952.

[32] Stoll, A. Zusammenhänge zwischen der Chemie des Chlorophylls und seiner Funktion in der Photosynthese. - Naturwiss., Jg. 24, H. 4, S. 53-59. 1936.

[33] Anson. M. L. On Lubimenko's Extracts of Chlorophyll-Protein — Science. Vol. 93, N 2308. 1941.

[34] Rabinowitch, E. Fotosintez (Photosynthesis), Vol. I.

[35] Sapozhnikov, D. I., I. A. Bronshtein, and G. A. Krasovskaya. Primenenie metoda bumazhnoi khromatografii dlya analiza pigmentov plastid zelenogo lista (Use of Paper Chromatography in Pigment Analysis of Plastids of Green Leaves). - Biokhimiya, 20(3):286-291. 1955.

[36] Nishimura, M. and K. Takamitsu. Studies on a Carotene-Protein Complex Isolated from Green Leaves. Plant physiol., Vol. 1, p. 305-310. 1960.

[37] For more details, see: Gyubbenet, E. R. Rastenie i khlorofill (Plant and Chlorophyll), p. 47-53.

[38] Apud: Serebryakov, K. K. Ocherki iz istorii botaniki (A Short History of Botany).— Leningrad, Uchpedgiz. 1951.

[39] Stoll, S. A. Zusammenhänge zwischen der Chemie. - Naturwiss., Jg. 24, H. 4, S. 53-59. 1936.

[40] Anson, M. L. On Lubimenko's Extracts of Chlorophyll-Protein.— Science, Vol. 93, N 2308, p. 186-187. 1941.

[41] Gyubbenet, E. R. Rastenie i khlorofill (Plant and Chlorophyll). 1951.

[42] Osipova, O. P. O belkovom komponente khlorofill-belkovogo kompleksa (The Protein Component of the Chlorophyll-Protein Complex). - Trudy Instituta Fiziologii Rastenii imeni Timiryazeva, 8(1):57-66. 1953.

[43] Smith, E. L. Solutions of Chlorophyll-Protein Compounds (Phyllochlorins) Extracted from Spinach. - Science, Vol. 88, N 2277, p. 170. 1938.

[44] Godnev, T. N. and O. P. Osipova. O prirode svyazi khlorofilla i belka v khloroplastakh (The Chlorophyll-Protein Bond in Chloroplasts). - Doklady AN SSSR, 55(2):161-164. 1947.
By the same authors: O sostoyanii khlorofilla v plastidakh i kharaktere svyazi pigmenta s belkovo-lipoidnoi stromoi khloroplasta (State of Clorophyll in Plastids and the Nature of the Pigment Bond with the Protein-Lipid Stroma of Chloroplasts). - Izvestiya AN SSSR, No. 1:17-26. 1948.
Osipova, O. P. O belkovom komponente khlorofill-belkovogo kompleksa (The Protein Component of the Chlorophyll-Protein Complex).- Trudy Instituta Fiziologii Rastenii AN SSSR, 3(1):57-66. 1953.
By the same author: K voprosu o sostoyanii khlorofilla v khloroplastakh (State of Chlorophyll in Chloroplasts). - Fiziologiya Rastenii, 4(1):28-32. 1957.

[45] Smith, E. L. The Chlorophyll-Protein Compound of the Green Leaf. - J. Gen. Physiol., Vol. 24, No. 5, pp. 565-582. 1941.

[46] Sapozhnikov, D. I. and T. G. Maslova. K voprosu o sostoyanii khlorofilla v list'yakh zelenykh rastenii (The State of Chlorophyll in Leaves of Green Plants). - Trudy Botanicheskogo Instituta AN SSSR, Seriya 4(11): 97-115. 1956.

[47] Neish, A. C. Studies on Chloroplasts. - Biochem. J., N 3, p. 293-299, 300-308. 1939.

[48] Rabinowitch, E. Fotosintez (Photosynthesis), Vol. 1.

[49] Krasnovskii, A. A. Fotosintez rastenii (Plant Photosynthesis). – Uspekhi Biologicheskikh Nauk, Vol. 1:473-506. 1950.
By the same author: Lyuminestsentsiya khlorofilla i fotosintez (Luminescence of Chlorophyll and Photosynthesis). – Biofizika, Vol. 1:3-18. 1959.

[50] Eisler, M. and L. Portheim. Über Fällungs Reaktionen in Chlorophyll und anderen Farbstofflösungen. – Biochem. Zs. Bd 130, S. 497-532. 1922.

[51] Noack, K. Der Zustand des Chlorophylls in der lebenden Pflanze. — Biochem. Zs., Bd 183, S. 135-152. 1927.

[52] Osipova, O. P. O svyazi khlorofilla s belkom (The Chlorophyll-Protein Bond). – Doklady AN SSSR, 57(4):371-374. 1947.

[53] Sapozhnikov, D. I. Poluchenie iskusstvennogo fitokhromoproteida (Synthetic Phytochromoprotein). – Doklady AN SSSR, 62(4):665-667. 1948.
Sapozhnikov, D.I. and T. G. Maslova. K voprosu o sostoyanii khlorofilla v list'yakh zelenykh rastenii (The State of Chlorophyll in Leaves of Green Plants). – Trudy Botanicheskogo Instituta AN SSSR, Seriya 4(11):97-115. 1956.

[54] Lepeschkin, W. W. Some Aspects of the State of Chlorophyll in Chloroplasts. – Plant Physiol., Vol. 24, N 1, p. 175-177. 1949.

[55] Rodrigo, F. A. Preliminary Note of Experiments Concerning the State of Chlorophyll in the Plant. – Biochem. et biophys acta, Vol. 10, N 2, p. 342. 1953.

[56] Noack, K. Photochemische Wirkung des Chlorophylls und ihre Bedeutung für die Kohlensäureassimilation. – Zs. Bot., Bd 17, S. 481-548. 1925.

[57] Seybold, A. and K. Egle. Blattpigmente. – Bot. Arch., Bd 41, S. 578-603. 1940.

[58] Rabinowitch, E. Fotosintez (Photosynthesis), Vol. 1.

[59] Sing, B. M. and N. K. Anantha Rao. State of Chlorophyll in Chloroplasts. — Current Sci., Vol. II, p. 442. 1942.

[60] Godnev, T.N., V.M. Terent'ev, and K.P. Parmon. O sravnitel'noi energii izvlecheniya khlorofilla raznykh rastenii neaktivnym rastvoritelyami (Comparative Energy of Chlorophyll Extracted from Various Plants by Inactive Solvents). – Sbornik nauchnykh trudov Instituta biologii AN BSSR, Vol. 1:3-7. 1950.

[61] Osipova, O. P. K voprosu o sostoyanii khlorofilla v khloroplastakh (The State of Chlorophyll in Chloroplasts). – Fiziologiya Rastenii, 4(1): 28-32. 1957.

[62] Brugger, J. E. and J. Frank. Experimental and Theoretical Contribution to Studies of Afterglow of Chlorophyll in Plant Materials. – Arch. Biochem. and Biophys., Vol. 75(2):465-496. 1958.

[63] Krasnovskii, A. A. and G. P. Brin. Opticheskie i fotokhimicheskie svoistva khlorofilla v razlichnykh vidakh svyazi (Optical and Photochemical Properties of Chlorophyll in Various Bond Systems). – Doklady AN SSSR, 63(2):163-165. 1948.

[64] Krasnovskii, A. A. Fotosintez rastenii (Plant Photosynthesis). – Uspekhi Biologicheskikh Nauk, Vol. 1:473-506. 1950.

[65] Krasnovskii, A.A. and L.M. Kosobutskaya. Spektral'nye issledovaniya sostoyaniya khlorofilla pri ego obrazovanii v rastenii i v kolloidnykh rastvorakh veshchestv etiolirovannykh list'ev (Spectral Studies on the State of Chlorophyll in Plants Upon Its Formation and in

Colloidal Solutions from Etiolated Leaves). — Doklady AN SSSR, 85(1):177-180. 1952.

By the same authors: Razlichnye sostoyaniya khlorofilla v list'yakh rastenii (Various States of Chlorophyll in Leaves). — Doklady AN SSSR, 91(2):343-346. 1953.

By the same authors: Deistvie rastvoritelei na spektral'nye svoistva i fotokhimicheskuyu aktivnost' khlorofilla v estestvennom sostoyanii (Effect of Solvents on the Spectral Properties and Photochemical Activity of Chlorophyll in Its Natural State). — Biokhimiya, 18(3):340-347. 1953

By the same authors: Aktivnaya forma khlorofilla v kolloidnykh rastvorakh veshchestva zelenykh list'ev i ee obratimye fotokhimicheskie prevrashcheniya (Active Form of Chlorophyll in Colloidal Solutions of Green Leaves and Its Reversible Photochemical Transformations). — Doklady AN SSSR, 104(3):220-443. 1955.

Krasnovskii, A.A. Uchastie pigmentov v reaktsiyakh fotosinteza rastenii (The Role of Pigments in Plant Photosynthesis). — Izvestiya AN SSSR, Seriya Biologicheskaya, No. 2:122-132. 1955.

By the same author: Lyuminestsentsiya khlorofilla i fotosintez (Luminescence of Chlorophyll and Photosynthesis). — Biofizika, 1(1):3-18. 1959.

By the same author: Fotokhimicheskii put' uchastiya pigmentov v reaktsiyakh fotosinteza (The Photochemical Course of Pigments in Photosynthetic Reactions). — In Sbornik: "Problemy fotosinteza", pp.30-43. Moskva. 1959.

Krasnovskii, A.A., E.A. Nesterovskaya and A.B. Gol'dberg. Spektral'noe issledovanie sostoyaniya khlorofilla v krasnoi vodorosli fillofora (Spectral Study of the State of Chlorophyll in the Red Alga Phyllophora). — Biofizika, 1(4):328-333. 1956.

Vorob'eva, L.M. and A.A. Krasnovskii. Fotokhimicheski aktivnaya forma khlorofilla i ee prevrashcheniya (The Photochemically Active Form of Chlorophyll and Its Transformations). — Biokhimiya, 21(1):126-136. 1956;

Krasnovskii, A.A., L.M. Vorob'eva and E.V. Pakshina. Issledovanie fotokhimicheski aktivnoi formy khlorofilla u rastenii razlichnykh sistematicheskikh grupp (Studies of the Photochemically Active Form of Chlorophyll in Plants of Various Taxonomic Groups). — Fiziologiya Rastenii, 4(2):124-133. 1957.

66 Godnev, T.N. and N.K. Akulovich. K voprosu o raspredelenii molekul khlorofilla v pigmentnom sloe granul khloroplastov (Distribution of Chlorophyll Molecules in the Pigment Layer of Chloroplast Granules). — Doklady AN SSSR, 120(6):1307-1310. 1958.

By the same authors: O raspolozhenii molekul khlorofilla v granulakh khloroplasta (Distribution of Chlorophyll in Chloroplast Granules). — Izvestiya AN SSSR, Seriya Fizicheskaya, 23(1):94-96. 1959;

Godnev, T.N. and R.V. Efremova. O spektrakh pogloshcheniya khlorofilla v zhivoi tkani lista (The Absorption Spectra of Chlorophyll in Intact Leaf Tissues). — Inzhenerno-Fizicheskii Zhurnal AN BSSR, 1(1):91-95. 1958.

67 Shlyk, A.A. and V. Prudnikova. Kinetychnyya asblivastsi ekstraktsyi khlorafilu e listsyau nepolyranymi rastvaral'nikami (Kinetic Characteristics of Chlorophyll Extracted from Leaves, by Nonpolar Solvents). — Vestsi Akad. Nauk Belarus. SSR, Seriya Biyalag., No. 3:16-21. 1958.

[68] Smith, J.H.C., K. Shibata, and R.W. Kart. A Spectrophotometric Accessory for Measuring Absorption Spectra of Light-Scattering Samples: Spectra of Dark Grown Albino Leaves and of Adsorbed Chlorophylls. — Arch. Biochem. and Biophys., Vol. 72, No. 2:457-464. 1957.

Smith, J.H.C., D.W. Kupke, J.E. Hoeffler, A. Behites, J. Ahrne, and A. Giese. The Natural State of Protochlorophyll. — Res. in Photosynthesis, pp. 464-474. New York. 1957.

[69] Shibata, K.Y. Spectroscopic Studies on Chlorophyll Formation in Intact Leaves. — J. Biochem. (Tokyo), Vol. 44, No. 3:147-173. 1957.

[70] Rabinowitch, E. Primary Photochemical and Photophysical Processes in Photosynthesis. — Discussions of the Faraday Soc., No. 27:161-172. 1959.

[71] Godnev, T.N. Osnovnye etapy biogeneza khlorofilla (Principal Stages in Chlorophyll Biogenesis). — In: Sbornik "Vozniknovenie zhizni na zemle", pp. 386-393. Moskva. 1957.

By the same author: Osnovnye etapy biogeneza khlorofilla (Main Stages in Chlorophyll Biogenesis). — Sbornik nauchnykh rabot Instituta biologii AN BSSR "Biokhimiya i fiziologiya rastenii", pp. 3-13. Minsk. 1958.

By the same author: Sovremennoe sostoyanie teorii obrazovaniya khlorofilla (Modern State of the Theory of Chlorophyll Synthesis). — In: Sbornik "Problemy fotosinteza", pp. 147-152. Moskva. 1959.

Godnev, T.N., R.V. Efremova, and L.A. Kravtsov. O spektral'nykh svoistvakh kompleksov khlorofilla i khlorofillida s belkami i nekotorymi drugimi soedineniyami (Spectral Properties of Chlorophyll and Chlorophyllid Complexes with Proteins and Some Other Compounds). — Trudy Instituta Fiziki i Matematiki AN BSSR, No. 2:85-92. 1957.

By the same authors: O prirode khlorofill-protein-lipoidnogo kompleksa pri ego obrazovanii iz protokhlorofilla (Nature of the Chlorophyll-Protein-Lipid Complex Ensuing from Protochlorophyll). — Doklady AN SSSR, Vol. 113:646-650. 1957.

By the same authors: O prirode khlorofill-proteinovogo kompleksa pri ego obrazovanii iz protokhlorofilla (Nature of the Chlorophyll-Protein Complex Ensuing from Protochlorophyll). — In: Sbornik "Problemy fotosinteza", pp. 163-168, Moskva. 1959.

Godnev, T.N. and Z.V. Khodasevich. O prirode khlorofilla v zhivykh plastidakh (Nature of Chlorophyll in Intact Plastids). — Byulleten' Instituta Biologii AN BSSR, No. 4:64-68. 1960.

[72] Okuntsov, M.M. Vliyanie medi na sostoyanie khlorofilla i starenie rastenni (Effect of Copper on the State of Chlorophyll and Aging of Plants). — Doklady AN SSSR, 54(9).837-840. 1946.

[73] Godnev, T.N. and O.P. Osipova. O sostoyanie khlorofilla v plastidakh i kharaktere svyazi pigmenta s belkovo-lipoidnoi stromoi khloroplasta (State of Chlorophyll in Plastids and the Nature of Its Bonds with the Protein-Lipid Stroma of the Chloroplasts). — Izvestiya AN BSSR, No. 1:17-26. 1948.

[74] Osipova, O.P. Ob izvlekaemosti khlorofilla iz zelenykh rastenii (On the Extractability of Chlorophyll from Green Plants). — Doklady AN SSSR, 54(8):799-801. 1947.

By the same author: O belkovom komponente khlorophyll-belkovogo kompleksa (The Protein Component of the Chlorophyll-Protein Complex).— Trudy Instituta Fiziologii Rastenii AN SSSR, 8(1):57-66. 1953.

Osipova, O.P. and I.V. Timofeeva. Issledovanie belka khloroplasta (Studies of the Protein of Chloroplasts). — Doklady AN SSSR, 67(1):105-108. 1949.

75 Maslova, T.G. Izuchenie sostoyaniya khlorofill-belkovo-lipoidnogo kompleksa v list'yakh v zavisimosti ot individual'nogo razvitiya i sistematicheskogo polozheniya rastenii (State of the Chlorophyll-Protein-Lipid Complex of Leaves in Relation to the Individual Development and Taxonomic Position of the Plant). — Author's Summary of Candidate Thesis, Leningrad. 1957.
By the same author: K voprosu o sostoyanii khlorofilla v plastidakh zelenykh rastenii (State of Chlorophyll in Plastids of Green Plants). — Botanicheskii Zhurnal SSSR, 43(1):103-106. 1958.

76 Godnev, T.N. and O.P. Osipova. O sostoyanii khlorofilla v plastidakh i kharaktere svyazi pigmenta (State of Chlorophyll in Plastids and the Character of the Pigment Bonds). —Izvestiya AN BSSR, No. 1. 1948.

77 Maslova, T.G. Izvlekaemost' khlorofilla petroleinym efirom i iz list'ev razlichnykh sistematicheskikh grupp (The Petroleum-Ether Extracts of Foliar Chlorophyll from Plants of Various Taxonomic Groups). — Botanicheskii Zhurnal SSSR, 44(3):389-393. 1959.

78 Gyubbenet, E.R. Rastenie i khlorofill (The Plant and Chlorophyll). — Izdatel'stvo AN SSSR, p. 24. 1952.

79 Timiryazev, K.A. Iskusstvennyi protofillin (Synthetic Protophyllin). Collected Works, Vol. 2: 264-267.
By the same author: Bestsvetnyi khlorofill (Colorless Chlorophyll). — Ibidem, pp. 268-269.
By the same author: Vosstanovlennyi khlorofill (Reduced Chlorophyll). — Ibidem, pp. 270-272.

80 Timiryazev, K.A. Protofillin v zhivom rastenii (Protophyllin in Living Plants). Collected Works, Vol. 2:273-275.
By the same author: Protofillin estestvennyi i protofillin iskusstvennyi (Natural and Synthetic Protophyllin). — Ibidem, pp. 276-279.

81 Monteverde, N.A. Über das Protochlorophyll. — Acta Horti Petrop, 1894, Bd. 13, S. 199-214;
By the same author: Vliyanie sveta na bystrotu obrazovaniya khlorofilla v list'yakh etiolirovannykh rastenii (Influence of Light on the Rate of Chlorophyll Synthesis in Leaves of Etiolated Plants). Trudy Sankt Peterburgskogo Obshchestva Estestvoispytatelei, 27(1):131-132. 1896.
By the same author: Protokhlorofill i khlorofill (Protochlorophyll and Chlorophyll). — Izvestiya Sankt Peterburgskogo Botanicheskogo Sada, 2(2):179-182, 1902;
By the same author: O spektre pogloshcheniya protokhlorofilla (Absorption Spectrum of Protochlorophyll). — Izvestiya Sankt Peterburgskogo Botanicheskogo Sada, 7(1)37-42, 47-58. 1907.

82 Timiryazev, K.A. Protofillin estestvennyi i protofillin iskusstvennyi (Natural and Synthetic Protophyllin). Collected Works, Vol. 2:276-279.

83 Liubimenko, V.N. Étude Spectroscopique des pigments verts des graines mûres: C.r. acad. sci., Paris, Vol. 42:1432-1435. 1906.

84 Monteverde, N.A. and V.N. Lyubimenko. O zelenom pigmente vnutrennei obolochki semyan nekotorykh Cucurbitaceae i ego otnoshenii k khlorofillu (The Green Pigment of the Inner Membrane of Some Representatives of Cucurbitaceae and Its Relation to Chlorophyll). — Izvestiya Sankt Peterburgskogo Botanicheskogo Sada, 9(2-3):27. 1909.

[85] Monteverde, N.A. and V.N. Lyubimenko. O zelenom pigmente (The Green Pigment). — Izvestiya Sankt Peterburgskogo Botanicheskogo Sada, 9(2 and 3):37. 1909.

[86] Liro, J.I. Über die photochemische Chlorophyllbildung bei den Phanerogamen. — Ann. Akad. Sci. Fennicae. Ser. A, t.1. 1908.

[87] Isachenko, B. L. Ob usloviyakh obrazovaniya khlorofilla (Prerequisites of Chlorophyll Synthesis). — Izvestiya Sankt Peterburgskogo Botanicheskogo Sada, 9(5):107. 1909.

[88] Monteverde, N.A. and V.N. Lyubimenko. Issledovaniya nad obrazovaniem khlorofilla u rastenii (Studies of Chlorophyll Synthesis in Plants). — Izvestiya AN SSSR, Seriya VI, 5(11): 1911.

[89] Monteverde, N.A. and V.N. Lyubimenko. Issledovaniya nad obrazovaniem khlorofilla u rastenii (Studies of Chlorophyll Synthesis in Plants). — Izvestiya Akademii Nauk, Seriya VI, 5(11):95. 1911.

[90] Schmidt, A. Die Abhängigkeit der Chlorophillbildung von der Wellenlänge des Lichtes. — Cohns Beiträge zur Biol. der Pflanzen, Bd. 12, H. 2:269-289. 1914.

[91] Greilach, H. Spectroscopische Untersuchungen über die Entstehung des Chlorophylls in der Pflanze. Sitzungsber. — Akad. Wiss. Wien, Mat.-nat. Cl., Bd. 113, Abt. 1:121-168. 1904.

[92] Gyubbenet, E.R. Rastenie i khlorofill (The Plant and Chlorophyll), pp. 89-91. — Izdatel'stvo AN SSSR. 1951.

[93] Gortikova, N.N. and D.I. Sapozhnikov. Kharakteristika temnovoi reaktsii zeleneniya (Chlorophyll Synthesis During the Dark Reaction). — "Sovetskaya Botanika", No. 5-6 :338-339. 1940.

[94] Godnev, T.N. Khlorofill i ego rol' v prirode (Chlorophyll and Its Role in Nature), p. 75. Minsk. 1955.

[95] Rabinowitch, E. Fotosintez (Photosynthesis), Vol. I, p. 407. [Russian translation. 1951.].

[96] Gyubbenet, E.R. Rastenie i khlorofill (The Plant and Chlorophyll), p. 94.

[97] Tsvet, M.S. Khromofilly v rastitel'nom i zhivotnom mire (Chromophylls in the Plant and Animal Kingdom), Warszawa, 1910.

[98] Willstätter, R. and A. Stoll. Untersuchungen über das Chlorophyll. Berlin, 1913.

[99] Noack, K. and W. Kiessling. Zur Entstehung des Chlorophylls und seiner Beziehung zum Blattfarbstoff. — Zs. physiol. Chem., Bd. 181: 13-49, 1929; Bd. 193:97-137. 1930.

[100] Seybold, A. Zur Kenntnis des Protochlorophylls. — Planta, Bd. 26, S. 712-718. 1937.

[101] Seybold, A. and K. Egle. Lichtfeld und Blattfarbstoffe. — Planta. Bd, 26:491-515, 1932; S. 87-123; 1938;
By the same authors. Zur Kenntnis des Protochlorophylls, Planta, Bd. 29;119-128. 1939.

[102] Krasnovskii, A.A. and K.K. Voinovskaya. Fotokhimicheskie svoistva protokhlorofilla (Photochemical Properties of Protochlorophyll). — Doklady AN SSSR, 55(4):663-666. 1949.

[103] Godnev, T.N. Khlorofill i ego rol' v prirode (The Role of Chlorophyll in Nature).

[104] Fischer, H., H. Mittenzwei and A. Oestreicher. Über Protochlorophyll und Vinylphäoporphyrin A_5. — Zs. physiol. Chem., Bd. 257:IV-VII, 1939;

Fischer, H. Fortschritte der Chlorophyllchemie. — Naturwiss., H. 26: 401-406. 1940.

[105] Krasnovskii, A.A., L.M. Kosobutskaya, and K.K. Voinovskaya. Aktivnye i neaktivnye formy protokhlorofilla, khlorofilla i bakteriokhlorofilla v fotosinteziruyushchikh organizmakh (Active and Inactive Forms of Protochlorophyll, Chlorophyll and Bacteriochlorophyll in Photosynthetic Organisms). — Doklady AN SSSR, 92(6):1201-1204. 1953.

[106] Rothemund, P. Protochlorophyll. Cold Spring Harbor Symposium on Qualitative Biology, Vol. V, N. 3, Cold Spring Harbor, pp. 71-79. 1935.

[107] Buslova, E.D. Novye dannye o protokhlorofille (Additional Data on Protochlorophyll). — In: Sbornik "Pamyati akademika V.N. Lyubimenko", pp. 27-42, Kiev. Izdatel'stvo AN SSSR. 1938.

[108] Godnev, T.N. Khlorofill i ego rol' v prirode (The Role of Chlorophyll in Nature).

[109] Godnev, T.N. K voprosu o posledovatel'nom formirovanii molekuly khlorofilla v rastenii (The Gradual Formation of Chlorophyll in Plants). — Dnevnik Vsesoyuznogo s"ezda botanikov v Moskve v yanvare 1926 goda, pp. 70-71. Moskva. 1926.

Godnev, T.N. and N.A. Naryshkin. K voprosu ob obrazovanii molekuly khlorofilla v rastenii v svyazi s sintezami soedinenii tipa tetrapirriletana (Formation of the Chlorophyll Molecule in Plants in Relation to the Synthesis of Tetrapyrilethane Compounds). — Nauchno-Agronomicheskii Zhurnal, No. 3:162-172. 1926.

[110] Monteverde, N.A.andV.N. Liubimenko. Recherches sur la formation de la chlorophylle chez les plantes. — Izvestiya Akademii Nauk, Seriya 6(5):73-100, Sankt-Peterburg, 1911.

Monteverde, N.A. and V.N. Lyubimenko. Issledovaniya nad obrazovaniem khlorifilla u rastenii. III soobschenie (Studies on Formation of Chlorophyll in Plants. 3rd Communication). — Izvestiya AN SSSR, Seriya 6(7):1007-1028. Sankt-Peterburg. 1913.

[111] Lyubimenko, V.N. O prevrashcheniyakh pigmentov plastid v zhivoi tkani rasteniya (Transformation of Plastid Pigments in Living Tissue of Plants). — Zapiski Akademii Nauk, Seriya 8, Vol. 33(12):1-274. 1916.

[112] Scharfnagel, W. Biologische Untersuchungen zur Chlorophyllbildung. — Planta, Bd. 13, H. 4:716-745. 1931.

[113] Noack, K. and W. Kiessling. Zur Kenntnis der Chlorophyllbildung. — Zs. angew. Chem., Jg. 44, N 5:93-96. 1931.

[114] Frank, R. The Effectiveness of the Spectrum in Chlorophyll Formation. — J. Gen. Physiol., Vol. 29:157-179. 1946.

[115] Krasnovskii, A.A. and L.M. Kosobutskaya. Spektral'noe issledovanie sostoyaniya khlorofilla pri ego obrazovanii v rastenii i v kolloidnykh rastovorakh veshchestva etiolirovannykh list'ev (Spectral Studies of the State of Chlorophyll Upon Its Synthesis in Plants and in Colloidal Solutions of Etiolated Leaves). — Doklady AN SSSR, 85(1): 177-180. 1952.

[116] For more details see: Godnev, T.N. Sovremennoe sostoyanie teorii obrazovaniya khlorofilla (Current Views on the Theory of Chlorophyll Synthesis). — In: Sbornik "Problemy fotosinteza", pp. 147-152.

[117] Rabinowitch, E. Fotosintez (Photosynthesis), Vol. 3:651. [Russian translation. 1959.]

[118] Godnev, T.N. Khlorofill i ego rol' v prirode (The Role of Chlorophyll in Nature), p. 74.

[119] Loc. cit.

[120] Shlyk, A. A. Metod mechenykh atomov v izuchenii biosinteza khlorofilla (Use of Labeled Atoms in the Study of Chlorophyll Biosynthesis). — Minsk, Izdatel'stvo AN BSSR, 1956.

[121] Godnev, T.N., A.A. Shlyk, and Ya.P. Lyakhnevich. O reaktsii perekhoda protokhlorofilla v khlorofill (Transformation of Protochlorophyll into Chlorophyll). — Fiziologiya Rastenii, 4(5):393-396. 1957.

[122] Rubin, B.A. Nekotorye zakonomernosti biosinteza khlorofilla (Some Patterns in the Biosynthesis of Chlorophyll). — In: Sbornik "Problemy fotosinteza", pp. 153-162.

[123] Lyubimenko, V.N. Ob obrazovanii khlorofilla v temnote (Chlorophyll Production in the Dark). — Izvestiya Sankt Peterburgskogo Botanicheskogo Sada, Vol. 5:195-202. 1905.

[124] Sachs, J. Übersicht der Ergebnisse der neueren Untersuchungen über das Chlorophyll. Flora. 1862.

[125] Famintzin, A. Die Wirkung des Lichtes auf das Ergrünen der Pflanzen.- Bull. Acad. sci. Bd. 10:548. Pétersbourg, 1866.

[126] Wiesner, J. Die Entstehung des Chlorophylls in der Pflanze. Wien, 1877.

[127] Greilach, P.H. Untersuchungen über die Entstehung des Chlorophylls in der Pflanze. Sitzungsber. — Akad. Wiss., Bd. 113, H. 3, Abt. 1: 121-168. 1904.

[128] Lyubimenko, V.N. O vliyanii napryazhennosti sveta na nakoplenie khlorofilla (The Influence of Light Intensity on Chlorophyll Accumulation). — Izvestiya Glavnogo Botanicheskogo Sada, Vol. 20:137-150. 1921.

[129] Lyubimenko, V.N. Ibidem, p. 149.

[130] Liubimenko, W. Observations sur la production de la chlorophylle chez végétaux supérieurs aux differentes intensités lumineuses. — C.r. acad. sci., Paris, t. 145:1347-1349. 1907.
Liubimenko, W. Production de la substance séche et de la chlorophylle chez les végétaux supérieurs aux differentes intensités lumineuses. — Ann. Sci. nat. Ser. t. 9. Botanique 7:321-415. 1909.

[131] Montéverdé, N.A.andV.N. Liubimenko. Recherches sur la formation de la chlorophylle chez les plantes. —Bull. acad. sci., Pétersbourg, ser. 6, t. 6, N 9:609-630. 1912.

[132] Gyubbenet, E.R. Rastenie i khlorofill (The Plant and Chlorophyll), pp. 115-116.

[133] Lyubimenko, V.N. and T.B. Forsh. K voprosu o fiziologicheskoi kharakteristike svetovykh i tenevykh list'ev (Physiological Properties of Leaves Grown in Light and in Darkness). — Izvestiya Nauchnogo Instituta imeni Lesgafta, Vol. 6:24-36. 1923.

[134] Lyubimenko, V.N. and V.A. Brilliant. Okraska rastenii (Plant Coloration), p. 122, Leningrad. 1924.

[135] Sachs, J. Wirkungen farbigen Lichtes auf Pflanzen. — Bot. Ztg, Jg. 20, N 47-49:353, 361, 369. 1864.

[136] Wiesner, J. Untersuchungen über die Beziehungen des Lichtes zum Chlorophyll. Sitzungsber. — Acad. Wiss. Wien, No. 69:324. 1877.

[137] Montéverdé, N.A.andV.N. Liubimenko. Recherches sur la formation de la chlorophylle chez les plants. — Izvestiya AN, Seriya 6(5): 449-498. 1911.

[138] Schmidt, A. Abhängigkeit der Chlorophyllbildung von der Wellenlänge des Lichtes. — Beitr. Biol. Pflanzen, Bd. 12:269-294. 1914.

[139] Lyubimenko, V.N. Fotosintez i khemosintez v rastitel'nom mire (Photosynthesis and Chemosynthesis in the Plant Kingdom). Moskva. 1935.

[140] Brandt, A.B., V.G. Derevyanko, I.P. Pavlova, and S.V. Tageeva. Znachenie razlichnoi intensivnosti i spektral'nogo sostava sveta dlya pigmentoobrazovanii rasteniyami (The Importance of Various Light Intensities and Spectral Composition for Pigment Production by Plants). — Biofizika, 2(6):649-659. 1957.

[141] Artari, A. Über Bildung des Chlorophylls durch grüne Algen. — Ber. Deutsch. bot, Ges., Bd. 20:201-207. 1902.

Artari, A. O vliyanii sredy na formu i razvitie vodoroslei (The Effect of Medium on Form and Development of Algae). Moskva. 1903.

[142] Burgerstein, A. Über das Verhalten der Gymnospermenkeimlinge im Lichte und im Dunkeln. Ber.- Deutsch. bot. Ges., Bd. 18:168-184. 1900.

[143] Bittner, K. Über Chlorophyllbildung im Finstern bei Kryptogamen. — Österr. Bot. Zs., S. 302-313. 1905.

[144] Sachs, J. Über das Ergrünen im Dunkeln. Flora, S. 505. 1864.

[145] Lyubimenko, V.N. Ob obrazovanii khlorofilla v temnote (Chlorophyll Synthesis in the Dark). — Izvestiya Peterburgskogo Botanicheskogo Sada, Vol. 5:195-202. 1905.

[146] Bulgakova, Z.P. and P.S. Engel'. Zelenenie khvoinykh v temnote i na svetu (Chlorophyll Synthesis by Conifers in the Dark and in Light). — Izvestiya Nauchnogo Instituta imeni Lesgafta. Vol. 15:177-182. 1929.

[147] Lyubimenko, V.N. Ob obrazovanii khlorofilla v temnote (Chlorophyll Synthesis in the Dark). — Izvestiya Peterburgskogo Botanicheskogo Sada, Vol. 5:198. 1905.

[148] Kuznetsov, N.I. Vvedenie v sistematiku tsvetkovykh rastenii (An Introduction to the Classification of Flowering Plants), pp. 75-92. Leningrad. 1936.

[149] Lyubimenko, V.N. O prevrashcheniyakh pigmentov plastid v zhivoi tkani rasteniya (Transformations of Plastid Pigments in Living Plant Tissues). — Zapiski AN Seriya 8 (Fiziko-Matematicheskaya), 33(12): 133-162. 1916.

[150] Schmidt, A. Clorophyllbildung in Konifernembryonen. — Bot. Arch., Bd.5, H.4, 1924.

[151] Yablokova, V.A. Okislitel'no-vosstanovitel'nyi potentsial v endosperme khvoinykh, razlichno zeleneyushchikh v temnote (The Redox Potential in Endosperm of Conifers Exhibiting Various Rates of Chlorophyll Synthesis in the Dark). — Doklady AN SSSR, 72(1):205-209. 1950.

[152] Godnev, T.N., A.A. Shlyk, and R.M. Rotfarb. O sinteze khlorofilla v temnote u pokrytosemyannykh rastenii (Chlorophyll Synthesis in Angiosperms in the Dark). — Fiziologiya Rastenii, 6(1):36-41. 1959.

[153] Lebedev, S.I. Fotosintez (Photosynthesis). — Kiev, Izdatel'stvo Ukrainskoi Akademii sel'skokhozyaistvennykh nauk, 1961.

[154] Sachs, J. Über den Einfluss der Temperatur auf das Ergrünen. Flora, 1864.

[155] Wiesner, J. Die Entstehung des Chlorophylls. Wien, 1877.

[156] Liro, J. Über die photochemische Chlorophyllbildung bei der Phanerogamen. — Ann. Akad. sci. Fennicae, ser. A.t. 1, 1908.

[157] Gyubbenet, E.R. Vliyanie temperatury na skorost' nakopleniya khlorofilla v etiolirovannykh prorostkakh (Influence of Temperature on the Rate of Chlorophyll Accumulation in Etiolated Seedlings). — Izvestiya Nauchnogo Instituta imeni Lesgafta, 11(1):39-56. 1925.

[158] Lyubimenko, V.N. and E.R. Gyubbenet. Vliyanie temperatury na skorost' nakopleniya khlorofilla v etiolirovannykh prorostkakh (Influence of Temperature on the Rate of Chlorophyll Accumulation in Etiolated Seedlings). — Izvestiya Nauchnogo Instituta imeni Lesgafta, 16(1, 2):165-188. 1930.
By the same authors: V.N. Liubimenko and E.R. Hubbenet. Influence of Temperature on the Rate of Accumulation of Chlorophyll in Etiolated Seedlings. — New Phytologist, Vol. 31:26-57. 1932.

[159] Gyubbenet, E.R. Rastenie i khlorofill (The Plant and Chlorophyll), p. 128.

[160] Lyubimenko, V.N. and E.R. Gyubbenet. Vliyanie temperatury na skorost' nakopleniya khlorofilla (Influence of Temperature on the Rate of Chlorophyll Accumulation), p. 183.

[161] Dement'ev, V. K voprosu ob obrazovanii i razlozhenii khlorofilla (Chlorophyll Production and Disintegration). — Byulleten' Moskovskogo Obshchestva Ispytatelei Prirody, 51(2):235-263. 1876.

[162] Timiryazev, K.A. Iskusstvennyi protofillin (Synthetic Protophyllin). Collected Works, Vol. 2:264-267;
By the same author: Bestsvetnyi khlorofill (Colorless Chlorophyll). — Ibid., pp. 270-272;
By the same author: Vosstanovlennyi khlorofill (Reduced Chlorophyll). — Ibid., pp. 268-269;
By the same author: Protofillin v zhivom rastenii (Protophyllin in Living Plants). — Ibid., pp. 273-275.

[163] Monteverde, N.A. O protokhlorofille (Protochlorophyll). — Izvestiya Peterburgskogo Botanicheskogo Sada, 13(2):179-182. 1894.
By the same author: Vliyanie sveta na skorost' obrazovaniya khlorofilla (Influence of Light on the Rate of Chlorophyll Synthesis). — Trudy Peterburgskogo Obshchestva Estestvoispytatelei, Vol. 27:131-132. 1896.
By the same author: Protokhlorofill i khlorofill (Protochlorophyll and Chlorophyll). — Izvestiya Peterburgskogo Botanicheskogo Sada, Vol. 2: 179-182. 1902.

[164] Palladin, V.I. Issledovaniya nad obrazovaniem khlorofilla v rasteniyakh (Studies on Chlorophyll Synthesis in Plants). — Trudy Obshchestva Estestvoispytatelei pri Varshavskom Universitete, Otdel Biologicheskii, 8(5):1-10. 1897.

[165] Scharfnagel, W. Biologische Untersuchungen zur Chlorophyllbildung. Planta, Bd. 13, H. 4:716-744. 1931.

[166] Norris, K.J. Observation on the Development of Chlorophyll and Carotenoid Pigments in Etiolated Plants. — Bull. Basic. Sci. Res. Univ. Cincinati, Vol. 5:23-33. 1933.

[167] Lyubimenko, V.N. and N.N. Gortikova. Pro rol' kysnyu v protsessi zeleneninnya (The Role of Oxygen in Chlorophyll Synthesis). — Zhurnal Instituta Botaniki Vseukrainskoi Akademii Nauk, No. 9:1-26. 1934.
By the same authors: O roli kisloroda v protsesse zeleneniya (The Role of Oxygen in Chlorophyll Synthesis). — Trudy Botanicheskogo Instituta AN SSSR, Seriya IV (Experimental botany), No. 2:113-148. 1936.

[168] Lyubimenko, V.N. and E.R. Gyubbenet. Vliyanie temperatury na skorost' nakopleniya khlorofilla v etiolirovannykh prorostkakh (Influence of Temperature on the Rate of Chlorophyll Accumulation in Etiolated Seedlings). — Izvestiya Nauchnogo Instituta imeni Lesgafta, 16(1, 2): 165-188. 1930.
By the same author: Influence of Temperature on the Rate of Accumulation of Chlorophyll in Etiolated Seedlings. — New Phytologist, Vol. 31:26-57. 1932.

[169] Lyubimenko, V.N. and N.N. Gortikova. O roli kisloroda v protsesse zeleneniya (The Role of Oxygen in Chlorophyll Synthesis ....), p. 133.

[170] Lyubimenko, V.N. O prevrashcheniyakh pigmentov plastid (On the Transformation of Plastid Pigments in Living Plant Tissues). — Zapiski AN, Seriya 8 (Fiziko-Matematicheskaya), 33(12): 133-162. 1916.

[171] Gortikova, N.N. Vliyanie temperatury na nakoplenie pigmentov plastid na svetu i v temnote (Influence of Temperature on Pigment Accumulation in Plastids in Light and in Darkness). — Uchenye Zapiski Leningradskogo Pedagogicheskogo Instituta, Vol. 25:137-145. 1939.

[172] Gortikova, N.N. and D.I. Sapozhnikov. Zavisimost' zeleneniya rastenii ot okislitel'no-vosstanovitel'nogo potentsiala (Dependence of Chlorophyll Synthesis on the Redox Potentital). — "Sovetskaya Botanika" No. 5:99-101. 1939.
By the same authors: Vliyanie okislitel'no-vosstanovitel'nogo potentsiala na zelenenie (Influence of the Redox Potential on Chlorophyll Synthesis). — Ibid., No. 5-6:338-339. 1940.

[173] Yablokova, V.A. Okislitel'no-vosstanovitel'nyi potentsial v endosperme khvoinykh, razlichno zeleneyushchikh v temnote (The Redox Potential in Endosperm of Conifers Exhibiting Various Rates of Chlorophyll Synthesis in the Dark). — Doklady AN SSSR, 72(1):205-209. 1950.

[174] Liubimenko, V.N. Recherches sur les pigments des plastes et sur la photosynthèse. III-e partie de la biologie de la photosynthèse. — Rev. gèn. bot., Vol. 40:146, 415, 486. 1928.

[175] Lyubimenko, V.N. Fotosintez i khemosintez v rastitel'nom mire (Photosynthesis and Chemosynthesis in the Plant Kingdom), Moskva. 1935.

[176] Roux, E. et Ch. Husson. Pigments des chloroplastes et photosynthèse. — C.r. acad. sci. Paris, Vol. 234, No. 15:1573-1575, 1952; Vol. 235, No. 19:1154-1574.

[177] Turchin, F.V., M.A. Gulizhskaya, and E.G. Plyshevskaya. O skorosti obnovleniya belka i khlorofilla v vysshikh rasteniyakh (Restoration Rate of Protein and Chlorophyll in Higher Plants). — Izvestiya AN SSSR, Seriya Biologicheskaya, No. 6:66-78. 1953.
Becker, R.S. and R.K. Sheline. Preliminary Experiments Using Mg^{28} as a Tracer with Chlorophylls, Plants and Their Extracts. — J. Chem. Phys., Vol. 25, No. 5:946-947. 1953.
Shlyk, A.A. Metod mechenykh atomov v izuchenii biosinteza khlorofilla (Use of Labeled Atoms in the Study of Chlorophyll Biosynthesis).

[178] Haberlandt, G. Vergleichende Anatomie des assimilatorischen Gewebesystems der Pflanzen. — Jb. Wiss. Bot., Bd. 13:74-188. 1882.

[179] Griffon, E. L'assimilation chlorophyllienne et la coloration des plantes. — Ann. sci. nat., 8 sér., Vol. 10, No. 1; 2:1-123. 1899.

[180] Liubimenko, W. La concentration du pigment vert et l'assimilation chlorophyllienne. — Rev. gén. bot., Vol. 20:162-177, 216-238, 253-267, 285, 297. 1908.

By the same author: Vliyanie sveta razlichnoi napryazhennosti na nakoplenie sukhogo veshchestva i khlorofilla u svetolyubivykh i tenevynoslivykh rastenii (Influence of Various Light Intensities on the Accumulation of Dry Matter and Chlorophyll in Photophilous and Sciophilous Plants). — Trudy po Lesnomu Opytnomu Delu, Vol. 13:1-110. 1909.

By the same author: Soderzhanie khlorofilla v khlorofil'nom zerne i energiya fotosinteza (Chlorophyll Content of the Chlorophyll Granules and Photosynthetic Energy). — Trudy Sankt Peterburgskogo Obshchestva Estestvoispytatelei, Vol. 41:1-266. 1910.

[181] Monteverde, N.A. and V.N. Lyubimenko. Issledovaniya nad obrazovaniem khlorofilla u rastenii. Tret'e soobshchenie. (Studies of Chlorophyll Synthesis in Plants. 3rd Communication). — Izvestiya Akademii Nauk, Seriya 6 (17):1007-1028.

[182] Liubimenko V.N. and E.R. Hubbenet. The Influence of Temperature on the Rate of Accumulation of Chlorophyll in Etiolated Seedlings. — New Phytologist, Vol. 31:26-57. 1932.

[183] Trudy Sankt Peterburgskogo Obshchestva Estestvoispytatelei, Vol. 45: 229-230. 1914.

[184] Lyubimenko, V.N. O prevrashcheniyakh pigmentov plastid v zhivoi tkani rasteniya (Transformations of Plastid Pigments in Living Plant Tissues). — Zapiski Akademii Nauk, Seriya 8, 33(12):198-199. 1916.

[185] Ibid, p. 202.

[186] Willstätter, R. and A. Stoll. Untersuchungen über das Chlorophyll. Berlin, 1913;

Willstätter, R. and A. Stoll. Untersuchungen über die Assimilation der Kohlensäure. Berlin, 1918.

[187] Apud Rabinowitch, E. Fotosintez (Photosynthesis), Vol. 1:425.

[188] Egle, K. Resistenz des Plastidenfarbstoffes. — Arch., Bd. 45, No. 1:93-148. 1944.

[189] Blagoveshchenskii, A.V. Biokhimicheskie osnovy evolyutsionnogo protsessa u rastenii (Biochemical Principles of Evolution in Plants), p. 103. — Izdatel'stvo AN SSSR. 1950.

[190] Lyubimenko, V.N. O kolichestve khlorofilla u morskikh vodoroslei (The Chlorophyll Content of Marine Algae). — Izvestiya Nauchnogo Instituta imeni Lesgafta. Vol. 10:127-132. 1924.

Liubimenko, V.N. Sur la quantité de la chlorophylle chez les algues marines. — C.r. Acad. sci., Paris, t. 179:1073-1076. 1924.

[191] Willstätter, R. and A. Stoll. Untersuchungen über das Chlorophyll.

[192] Seybold, A. and K. Egle. Quantitative Untersuchungen über Chlorophyll und Carotinoide der Meeresalgen.

[193] Engelmann, Th.W. Farbe und Assimilation. — Bot. Ztg., Jg. 41, 1883, S. 18;

By the same author. Untersuchungen über die quantitativen Beziehungen zwischen Absorption des Lichtes und Assimilation in Pflanzenzellen. — Bot. Ztg, Jg. 42:23, 349. 1884.

[194] Oltmanns, F. Morphologie und Biologie der Algen. Jena, 1905.

[195] Harder, R. Über die Bedeutung von Lichtintensität und Wellenlänge für die Assimilation farbiger Algen. — Z. Bot., Bd. 15:305. 1923.

[196] Lyubimenko, V.N. K voprosu o khromaticheskoi adaptatsii (Contribution to Chromatic Adaptation). — Izvestiya Nauchnogo Instituta imeni Lesgafta, 12(1):5-30. 1926.
By the same author: O pigmentakh plastid v svyazi s khromaticheskoi adaptatsii (The Plastid Pigments in Relation to Chromatic Adaptation). — Dnevnik Vsesoyuznogo s"ezda botanikov v yanvare 1926 goda (Reports of the All-Union Conference of Botanists in January 1926), p. 117, Moskva. 1926.

[197] Rabinowitch, E. Fotosintez (Photosynthesis), Vol. 1:424.

[198] Liubimenko, V.N. Recherches sur les pigments des plastes et sur la photosynthèse. III. La biologie de la photosynthèse. — Rev. gén. bot., t. 40:415-447, 486-512. 1928.

[199] Lyubimenko, V.N. and Z.P. Tikhovskaya. Opyty nad fotosintezom u morskikh zelenykh, burykh i krasnykh vodoroslei v svyazi s izucheniem khromaticheskoi adaptatsii (Experiments on Photosynthesis of Green, Brown and Red Marine Algae in Relation to Investigations of Chromatic Adaptation). — Dnevnik Vsesoyuznogo s"ezda botanikov v Leningrade v yanvare 1928 goda (Reports of the All-Union Conference of Botanists in Leningrad, January 1928), pp. 40-41. Leningrad. 1928.
Liubimenko, V.N. and Z.P. Tihovskaja. Recherches sur la photosynthèse et l'adaptation chromatique chez les algues marines. — Trudy Sevastopol'skoi Biologicheskoi Stantsii AN SSSR, Vol. 1:153-190. 1929.

[200] Lyubimenko, V.N. O khlorofille v otlozheniyakh ozernogo ila (Chlorophyll in Lacustrine Silt Deposits). — Zhurnal Russkogo Botanicheskogo Obshchestva, Vol. 6:95-105. 1921.
By the same author: Pro kopalyni reshetky khlorofilu u vidkladakh mors'kogo mulu (On Fossil Remnants of Chlorophyll in Marine Ooze (or Silt)). — Zapiski Fizyko-Matematychnoho Vydavnytstvo Vseukrayinskoyi Akademiyi Nauk, 4(5):351-356. 1930.
Liubimenko, V.N. and Mme Rauser-Cernoussova. Sur les restes fossiles de la chlorophylle dans les sediments limoneux marins. — C.r. Acad. sci., Paris, t. 190:813-815. 1930.

[202] Lyubimenko, V.N. O khlorofille v otlozheniyakh... (Chlorophyll in Lacustrine Silt Deposits), p. 101.

[203] Lyubimenko, V.N. and A.I. Palamarchuk. Kolichestva khlorofilla kak nasledstvennyi priznak u Nicotiana tabacum L. (Chlorophyll Amount a Hereditary Features in Nicotiana tabacum L.). — Trudy Byuro po Prikladnoi Botanike, Vol. 9:463-478. 1916.
By the same authors: Kolichestvo khlorofilla kak nasledstvennyi priznak u Nicotiana tabacum L. (Chlorophyll Amount a Hereditary Feature of Nicotiana tabacum L.). — Zhurnal Russkogo Botanicheskogo Obshchestva, Vol. 8:24-31. 1923.

[204] Correns, C. Vererbungsversuche mit blass-(gelb)-grünen und bunt blättrigen Sippen bei Mirabilis jalapa, Utrica pilulifera und Linaria annua. — Zs. Indikative Abstammungs- und Vererbungslehre, Bd. 4: 81-101. 1911.

[205] Baur, E. Untersuchungen über die Vererbung der Chromatophorenmerkmalen bei Melandrium, Antrrhinum and Aquilegia. — Zs, Indikative Abstammung und Vererbungslehre, Bd. 4:81-101. 1911.

[206] Lyubimenko, V.N. and V.A. Brilliant. Okraska rastenii (Coloration of Plants), p. 137, Leningrad. 1924.

[208] Breslavets, L.P. O nasledstvennosti okraski venchika Tropaeolum majus L. (Inheritance of the Corolla Coloration of Tropaeolum majus L.). — Izvestiya Nauchnogo Instituta imeni Lesgafta, Vol. 3:63-68. 1921.

[209] Lyubimenko, V.N., O.A. Shcheglova, and N.N. Gortikova. O fiziologicheskikh osnovakh nasledovaniya okraski plastid. Kolichestvo khlorofilla, ksantofilla, karotina i nikotina u mezhvidovykh gibridov roda Nicotiana (The Physiological Basis of the Inheritance of Plastid Pigmentation. The Amount of Chlorophyll, Xanthophyll, Carotene and Nicotine in Interspecific Hybrids of Genus Nicotiana). — Izvestiya Nauchnogo Instituta imeni Lesgafta, 20(2):3-36. 1937.

[210] Lyubimenko, V.N., O.A. Shcheglova, and N.N. Gortikova. O fiziologicheskikh osnovakh nasledovaniya okraski plastid (Physiological Basis of the Inheritance of Plastid Pigmentation). — Izvestiya Nauchnogo Instituta imeni Lesgafta, 20(2):31-32. 1937.

EXPLANATORY LIST OF ABBREVIATIONS OF USSR INSTITUTIONS AND PERIODICALS APPEARING IN THIS TEXT

Abbreviation	Full name (transliterated)	Translation
AMN	Akademiya Meditsinskikh Nauk	Academy of Medical Sciences
AN SSSR	Akademiya Nauk SSSR	Academy of Sciences of the USSR
BSSR	Belorusskaya Sovetskaya Sotsialisticheskaya Respublika	Belorussian Soviet Socialist Republic
Mosk. Obshch. Ispyt. Prir.	Moskovskoe Obshchestvo Ispytatelei Prirody	Moscow Society for Natural Research